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(Continued)

EFFECT OF X-RAY RADIATION ON HYPERSENSITIVENESS*†‡

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INTRODUCTION

EXPERIMENTAL hypersensitiveness includes anaphylaxis and precipitin formation, the Arthus phenomenon, the Schultz-Dale reaction, and diffuse vascular or collagen disease. This latter condition has been produced by sensitizing rabbits (horse serum and toxin)[2-7, 10-12, 16] but may be produced also by nonspecific methods.[1, 8, 9, 13-15] There is evidence that most of these phenomena are based on an immunologic process, namely, an antigen-antibody reaction. Since antibodies are probably formed by the reticuloendothelial system, it therefore follows that blocking of or interference with the function of the reticuloendothelial system will affect antibody formation. Such decrease in the production of antibodies should, therefore, have a direct effect upon the development of any of the reactions of hypersensitiveness. This may conceivably shed some light on the mechanism of some collagen diseases in man since it appears that some of these are related to hypersensitiveness.[17-23]

Site of Antibody Formation.—Numerous sites of antibody production have been suggested. It is believed that the reticuloendothelial system which includes the liver, spleen, bone marrow, and lymph nodes is the source of antibody formation. Recently, attention has been focused on the role of the lymphocyte in antibody production. McMaster and Hudack[25] have shown that agglutinins are formed within lymph nodes. A "neutralizing principle" for vaccinia has been demonstrated as arising from the lymph nodes by McMaster and Kidd.[26] Kass[27] and Ehrich and Harris[28] concluded that lymphocytes actually contain gamma globulin. It has also been shown that lymph obtained from the regional lymphatics which drain the site of antigen injection contain a significant antibody titer sooner than could be demonstrated in the peripheral blood.[29-31] This has been questioned by Habel, Endicott, Bell, and Spear[32] who stated that there is very little significant experimental evidence to indicate that lymphocytes and lymph

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nodes carry large amounts of antibody. Harris, Rhoads, and Stokes[33] found that the thymus does not play a demonstrable role in antibody production and that there is only slight evidence of the formation of antibodies in the spleen when antigen is injected subcutaneously. However, when antigen is injected intravenously, high titers were noted in the splenic extracts. This variation in antibody titer by the various organs of the reticuloendothelial system caused by different routes of administration was further proven by Roberts, Adams, and White.[34] These authors found that the administration of antigen intravenously causes a greater titer of antibody in the spleen and an absent titer in the lymph node and thymus extracts. When the antigen is given intraperitoneally the spleen shows no titer but the lymph nodes show a very high titer.[34] Thus, the route of administration of the antigen may also play a role in the site of antibody formation. There is additional experimental evidence[35, 36] which indicates that the plasma cell may also play a definite role in antibody production.

Interference with Function of Antibody-Forming Organs.—Various methods have been used for the destruction of the sites of antibody formation. Graef, Karnofsky, Jager, Krichesky, and Smith[37] have shown that the administration of nitrogen mustard to experimental animals is followed by a fall in the circulating polymorphonuclear leukocytes, lymphocytes, and erythrocytes. Utilizing this knowledge, it has been demonstrated that the administration of nitrogen mustard is followed by a diminution in antibody production.[15, 38-40] Dammin and Bukantz[16] noted an inhibition of the production of the Arthus phenomenon and the diffuse vascular lesions of hypersensitivity as well as the precipitin titers by this method. Other agents may interfere with some immunologic reactions. Kyser, McCarter, and Stengle[41] inhibited serum-induced myocarditis in rabbits by the use of antihistaminics. However, Roberts, Crockett, and Laipply[42] found that Benadryl did not prevent the vascular and myocardial lesions of the anaphylactic type, but apparently did prevent, to some extent, the development of the pericardial, endocardial, and valvular lesions. Dammin and Bukantz[16] also showed that the antihistaminics have no demonstrable effect on antibody production, the in vitro antigen-antibody combination, or the development of Arthus reactions and vascular lesions. These latter investigators as well as others[41-46] have shown that salicylates may possibly exert some degree of inhibition in the antigen-antibody reaction as shown by precipitin titers and vascular lesions. Forman, Seifter, and Ehrich[45, 46] showed that Dicumarol caused inhibition of the in vitro antigen-antibody combinations, that colchicine caused depression of the precipitin titer and also abolished the allergic arteritis.

Previous investigators[47-54] have shown that x-ray exerts a definite depressant effect upon the production of antibodies. Becker[24] reported a complete depression of the Schwartzman phenomenon with 800 r of x-ray, nitrogen mustard, and benzol. In previous experiments on rabbits, it has been shown that 600 to 800 r, the latter being the LD_{50}, cause destruction of the lymph nodes with complete regeneration of lymphocytes in 21 days, destruction of the splenic follicles with regeneration complete in 14 days, and destruction of bone marrow with

complete regeneration at the end of 21 days.[55] It was also noted by other investigators[53, 54] that even small dosages of x-ray cause a fall in the total peripheral lymphocyte count. Craddock and Lawrence[53] noted that irradiation with 250 r causes a leukopenia lasting 30 to 40 days. This indicates, then, that x-ray radiation has a very damaging effect on the lymphoid system. However, not all the cellular elements of the blood are destroyed even at 800 r for 24 hours following irradiation, the macrophages and reticulum cells are not affected, and in 9 days plasma cells are found in the lymph nodes.[55]

EXPERIMENTAL DATA

This paper deals with our experience in the evaluation of the effect of x-ray radiation on the development of diffuse vascular disease (collagen disease), the Arthus phenomenon, the Schultz-Dale reaction, and the production of precipitins in sensitized animals.

Forty normal male rabbits, each weighing approximately 2 kilograms, were used in this study. They were divided as follows: Eight were sensitized with horse serum. These animals were not exposed to x-ray radiation and, therefore, used as controls. Twenty-eight were irradiated prior to sensitization. Four were irradiated without any attempt at sensitization so as to demonstrate the effect of x-ray alone on the normal rabbits.

The "Coca technique" of sensitization as will be seen below was used in one group of animals because it yields most readily a high precipitin titer. The "Rich technique," however, yields a high precipitin titer as well as pronounced vascular and myocardial lesions and, therefore, is the method of choice for this study.

Effect of Sensitization by "Coca Technique" Without Previous X-ray Radiation

Group I.—This group consisted of 4 rabbits which received horse serum* for the optimum demonstration of precipitins.[56] The method consisted of the subcutaneous injection of 1.0 c.c. of horse serum on the first, fifth, and eighth days. From the ninth day on, the rabbits were given 0.2 c.c. of horse serum intraperitoneally every other day for 7 injections. The animals were then sacrificed by air embolus on the twenty-sixth day. This technique was used again in Group III and will be termed the "Coca technique." The animals sensitized by this method revealed the following:

Vascular lesions.—Study of microscopic sections from the "Coca treated" animals revealed no evidence of diffuse vascular changes or of myocardial disease.

Arthus phenomenon.—This was observed in 3 of 4 sensitized animals. It was produced by the intradermal injection of 1.0 c.c. of normal horse serum into a shaved site on the skin of the back of the rabbit, 12 to 14 days after sensitization. The reaction consisted of a hemorrhagic response in the injected site proceeding to necrosis within 24 to 48 hours.

Schultz-Dale reaction.—The Schultz-Dale reactions were studied in this group and all other subsequent groups of sensitized animals. The muscle strips were taken immediately after death and were suspended in Tyrode solution.

*The normal unpreserved horse serum was supplied through the courtesy of Lederle Laboratories, Division of the American Cyanamid Co., New York, N. Y.

To the bath was added 0.1 c.c. to 0.4 c.c. of normal horse serum. Animals sensitized by both the Coca and Rich techniques were studied in this manner. In this group the intestinal strip of one animal showed a positive Schultz-Dale reaction and in the remaining 2, a negative reaction was observed. These results will be interpreted later.

Precipitins (Table I).—Precipitin tests were done in all groups of animals according to two different methods. One method was that of the ring test.[57] With a capillary pipette, normal horse serum in varying dilutions was layered in precipitin tubes above a constant amount of rabbit serum, so as to provide a ring contact. The tubes were examined for the appearance of a distinct ring in 20 ot 30 minutes with the highest dilution at the point of contact as the final reading. The second method employed was that of a serum dilution technique,[58] in which 0.2 c.c. of horse serum diluted 1:5 with physiologic saline was mixed with 1.0 c.c. of rabbit serum in varying dilutions. The tubes were incubated for one hour at room temperature and then placed in a refrigerator. They were observed for the formation of a precipitate at the end of 24 and 48 hours. The tube containing the highest dilution of rabbit serum showing the precipitate was taken as the final titer reading. The rabbit antisera were studied by both of these methods for their precipitin titer. The serum precipitin titers of the 4 control animals in this series showed the following: (a) with the antigen dilution technique, 2 animals showed titers of 1:1000 and 2 showed titers of 1:10,000; and (b) in the serum dilution technique, two showed 1:100, one 1:200, and another 1:1000.

TABLE I. PRECIPITIN TITERS

TECHNIQUE	RABBIT NUMBER	RING TEST. HORSE SERUM ANTIGEN DILUTION	RABBIT SERUM ANTIBODY DILUTION
Coca control	16	1:10 000	1:100
	22	1:1,000	1:200
	5	1:1,000	1:100
	23	1:10,000	1:1,000
Coca x-ray	49	1:10	-
	48	1:10	1:10
	46	1:10	1:100
	14	1:10	1:5
	15	0	0
	11	1:10	1:20
Rich control	7	1:10,000	1:500
	18	1:10,000	1:500
	19	1:2,000	1:100
	24	1:2,000	1:200
Rich x-ray	77	1:4,000	1:100
	80	1:500	1:20
	72	1:100	1:20
	73	1:1,000	1:20
	74	1:50	1:20
	76	1:500	1:500
	6	0	0
	84	1:400	1:20
	85	0	0

Effect of Sensitization by "Rich Technique" Without Previous X-ray Radiation

Group II.—This group consisted of 4 animals sensitized according to the technique of Rich and Gregory[2] in order to demonstrate the experimental pro-

duction of vascular lesions as well as other phenomena of hypersensitivity as indicated below. None of these animals were exposed to x-ray radiation. This method of sensitization consisted of the injection of 10 c.c. of horse serum per kg., as a sensitizing dose, and the subsequent administration of 1.0 c.c. of horse serum on the seventeenth day. On the nineteenth day, 10 c.c. of horse serum per kg. were again given and the animals were sacrificed by air embolus on the twenty-sixth day. An Arthus phenomenon was performed on the 12-24th day. This technique was used subsequently in Group IV and will be termed the "Rich technique."

Sections of the heart, lungs, spleen, liver, pancreas, adrenals, and kidneys were examined after fixation in formalin and staining with hemotoxylin and eosin.

Pathologic study of microscopic sections revealed the following: The lesions included perivascular infiltration of blood vessels with lymphocytes, plasma cells, and eosinophiles. The myocardium showed infiltration with plasma cells and lymphocytes with myocardial degeneration. The lesions seen on microscopic study of the tissues of these animals were graded according to the following criteria: none—no lesions seen; very slight (1+)—one or two small venules showing perivascular infiltration usually only in the lung; slight (2+)—when only a minor infiltration of round cells was seen in the myocardium plus one or two venules showing perivascular round cell infiltration of the lungs alone; moderate (3+)—when two or three lesions of round cell infiltrations were observed in one section or only two lesions seen in two or three sections; marked (4+)—lesions showing extensive infiltration of round cells in the myocardium and extensive perivascular infiltration in the viscera.

Vascular lesions.—Pathologic examinations of this group of 4 animals showed extensive (4+) lesions in all the animals. The lesions were characteristic of periarteritis nodosa and rheumatic carditis as described by Rich and Gregory.[2] (Figs. 1 and 2.)

Arthus Phenomenon.—In this group, positive Arthus phenomenon was elicited in 3 of 4 animals. The fourth showed a doubtful reaction.

Schultz-Dale reactions.—This reaction was negative in 2 and doubtful in one animal in the group of 3 rabbits studied (Table II). This would suggest that this test for fixed antibody is not quite reliable with the rabbit ileum, although more animals are necessary for statistical facts.

TABLE II. SUMMARY OF FINDINGS IN SENSITIZED RABBITS, WITH AND WITHOUT PREVIOUS EXPOSURE TO X-RAY RADIATION

	RABBITS SENSITIZED TO HORSE SERUM (CONTROL)	RABBITS RECEIVING 600 R TO 800 R X-RAY 24 TO 72 HOURS PRIOR TO SENSITIZATION WITH HORSE SERUM
Schultz-Dale reaction	Inconclusive	Inconclusive
Arthus phenomenon	Positive	Negative
Serum precipitin titers	High	Depressed
Vascular and myocardial lesions in internal organs	Marked	Absent to Slight
White blood counts	Normal	Lymphopenia

Precipitins (Table I).—A study of the precipitin titers showed 2 animals with titers of 1:10,000 and 2 with 1:2,000 with the antigen solution technique. With the antibody dilutions, 2 showed 1:500, one 1:200, and another 1:100.

Effect of X-ray Radiation Previous to Sensitization by the "Coca Technique"

Group III.—Six rabbits were sensitized to horse serum according to the technique of Coca.[56] X-ray radiation was given to 3 of these animals with 600 r and in 3 others with 800 r 24 hours prior to the first sensitizing dose of normal horse serum. In all animals subjected to x-ray radiation in these experiments the method of exposure was as follows: a single dose of 600 r or 800 r of x-ray (200 K.V., 15 M.A., aluminum 1 mm., copper 0.5 mm. filter, output 47 r per minute for 12.75 minutes or 17.02 minutes, focal skin distance 56 cm.). The x-ray radiation was given 24 to 72 hours before sensitization.

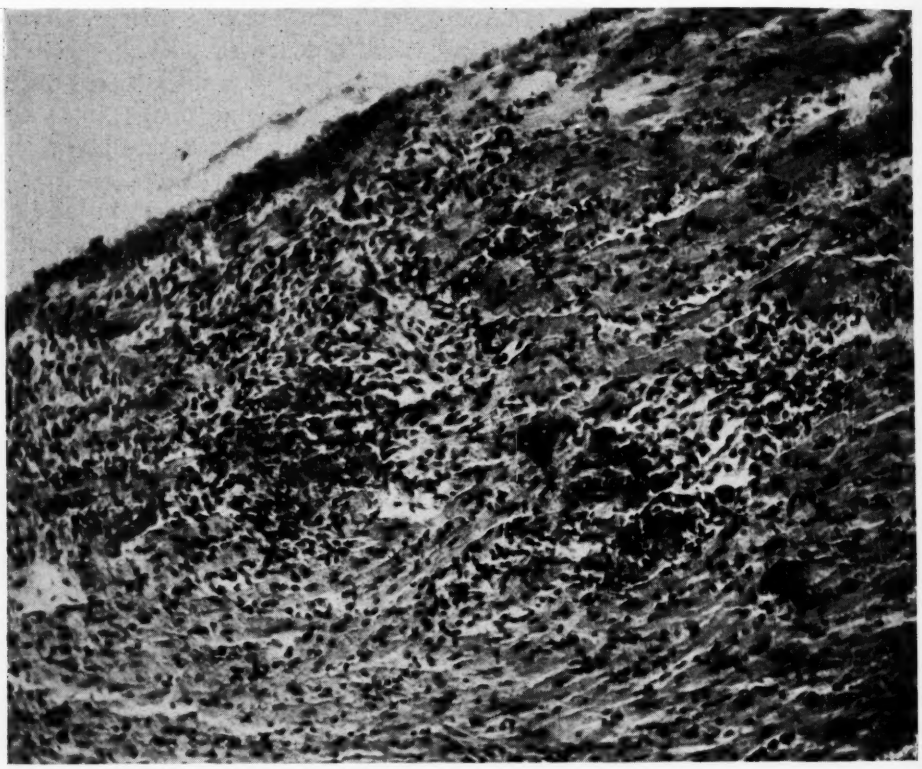

Fig. 1.—Rabbit myocardium in which the "Rich technique" of sensitization was used shows infiltration with lymphocytes, plasma cells, myocardial degeneration, and giant cell formation. (×125.)

Vascular lesions.—Lesions were found in none of the animals in this group.

Arthus phenomenon.—The 6 rabbits thus treated showed a negative reaction when an attempt was made to elicit the Arthus phenomenon.

Schultz-Dale reaction.—In 3 animals that received 800 r of x-ray prior to sensitization, the Schultz-Dale reaction was negative with intestinal strip.

Precipitins (Table I).—In the 6 rabbits that had been exposed to 500 r or 800 r of x-ray radiation prior to sensitization with normal horse serum, there

was a definite depression of the serum precipitin titer, according to both the antigen and antibody dilution techniques. With the antigen-dilution technique one animal showed no titer, and the 5 others showed titers of 1:10. Using the antibody-dilution technique one animal showed no titer, one showed a titer of 1:5, two 1:10, one 1:100, and there was one animal in which the serum was not titrated.

Fig. 2.—Section of rabbit lung in which the "Rich technique" of sensitization was used shows a vessel with hyperplasia of the intima and infiltration of the perivascular tissue with lymphocytes and plasma cells. (×125.)

Effect of X-ray Radiation Previous to Sensitization by the "Rich Technique"

Group IV.—This group consisted of 22 rabbits which were sensitized to horse serum according to the method of Rich and Gregory,[2] having previously been treated with 600 r to 800 r of x-ray radiation. Fifteen rabbits received 600 r of x-ray radiation and 7 received 800 r, 24 to 72 hours prior to sensitization with normal horse serum. The animals died or were sacrificed from the fourth to the twenty-eighth day after receiving horse serum. Of the 22 animals, Arthus phenomena were done on 15; 7 were tested for the Schultz-Dale reaction and 9 animals gave precipitin tests.

Vascular lesions.—Pathologic examination of the tissues of these animals yielded the following findings: Of the 22 animals, 8 showed no lesions whatsoever, 4 showed very slight (1+) lesions, 8 showed only slight (2+) lesions, and 2 showed moderate (3+) lesions. No animals showed lesions of the severity (4+) of the sensitized nonirradiated controls (Group II). No lesions were found in the liver, spleen, or kidneys. The pancreas and the adrenal glands were also free from vascular lesions. The lesions that were found consisted of minimal infiltration of myocardium with lymphocytes and plasma cells. The lesions seen in the lungs were primarily those of perivascular infiltration of lymphocytes and plasma cells surrounding small venules.

Arthus phenomenon.—In these 22 rabbits subjected to x-ray radiation prior to sensitization with normal horse serum, the Arthus phenomena was done in 15. All of these were negative. This would seem to indicate that x-ray radiation had sufficiently impaired the formation of antibodies below the level necessary to produce this lesion of hypersensitiveness.

Schultz-Dale reaction.—Seven of the animals were tested for the Schultz-Dale reaction. Four showed contraction of the intestinal strip when horse serum antigen was added to the bath. In 3, negative results were obtained. These results will be interpreted later in the paper.

Precipitins (Table I).—In 9 animals subjected to 600 r and 800 r of x-ray radiation prior to sensitization with horse serum, there was definite depression of the precipitin titer (except in one case) when compared to the control group receiving horse serum alone. The results were obtained with both the antigen-dilution and antibody-dilution precipitin test techniques. Two showed no titer at all, 2 showed titers of 1:50, one 1:100, one 1:400, one 1:500, one 1:1000, and one 1:4000 using the antigen-dilution method. With the antibody-dilution method 2 showed no titers, five 1:20, one 1:100, and another 1:500.

Effect of X-ray Radiation on Normal Nonsensitized Animals (Control)

Group V.—Four rabbits were used in this control group. These had x-ray radiation alone without any sensitization to horse serum. Three received 600 r and one received 800 r. No significant vascular or cardiac lesions were found in any of the microscopic studies of the internal organs of the animals in this group.

Total white blood counts done at weekly intervals reveal that the x-rayed rabbits developed a leukopenia with an absolute decrease in lymphocytes. The total white blood counts in this group ranged between 1,400 to 2,300 with lymphocytes varying between 20 to 50 per cent. In the control group of normal rabbits sensitized to horse serum alone without prior x-ray radiation, the total white counts ranged between 7,000 to 12,500 with lymphocyte counts varying between 52 to 88 per cent.

DISCUSSION

Histologic sections of the organs of sensitized animals previously exposed to x-ray show as indicated above a marked reduction in the number and severity of vascular lesions in comparison with the control group of nonirradiated but sensitized animals. This indicates that x-ray radiation interferes with the

mechanism of production of these lesions. X-ray radiation in the dosages of 600 r and 800 r did not completely destroy all the cellular elements of the reticuloendothelial system. Higher dosages were not used because 800 r is considered to be the LD_{50} dose.

The fact that rabbits treated with x-ray previous to sensitization did not develop an Arthus phenomenon, as contrasted with the control sensitized group that were not subject to x-ray radiation, suggests possibly that x-ray exerts a depressive effect upon the formation of antibodies upon which this phenomenon depends. Since the Arthus phenomenon is probably dependent on a certain level of antibody content, it may then be reasonable to assume that x-ray radiation produces a depressant effect upon the formation of antibodies below the critical level necessary for the production of this phenomenon. This does not result from the nonspecific effect of x-ray on tissues since antibody titers were also concomitantly depressed. A review of the microscopic sections of the animals which received x-ray radiation alone showed no lesions and thus the nonspecific effect of x-ray radiation can be ruled out.

The Schultz-Dale reaction in the rabbits proved too inconclusive in our hands. This agrees with the opinion of Chase[59] and Mayer[60] who suggest that the rabbit ileum has a lower index of reactivity than the uterine strip of the guinea pig which is employed in the standard method of testing for smooth muscle antibody by the Schultz-Dale technique. Several sensitized rabbit intestinal strips in the control group that had received no x-ray showed no muscular contractions on the addition of horse serum antigen into the Schultz-Dale bath. In the group of sensitized animals who had received x-ray, some showed positive smooth muscle contractions with this test. This is particularly difficult to interpret in view of the fact that their serum precipitin titers were lower than the controls, the vascular lesions were less extensive than those seen in the control animals, and, finally, because the Arthus phenomena was absent in these animals. The reason for these findings is not clearly understood, unless it can be explained on the basis of a lower degree of reactivity of the sensitized rabbit ileum. This would indicate again that the rabbit ileum is unsatisfactory for the performance of the Schultz-Dale technique.

With few exceptions, the precipitin titers of the serum of the irradiated sensitized animals were consistently lower than that of the control group. This was evidenced by both the antigen dilution and the serum dilution techniques done on the serum of rabbits which were sensitized according to both the methods of Coca, Walzer, and Thommen[56] and Rich and Gregory.[2] This fact also suggests that x-ray radiation exerts a depressive effect upon the formation of antibodies but does not completely inhibit their formation since in the doses and time intervals we used (Table II), it appears to act chiefly on the lymphoid element of the reticuloendothelial system.

At dosages of x-ray as high as 600 r and 800 r, the cells of the reticuloendothelial system were completely regenerated before the completion of our experiments. For example, the lymphocytes in the lymph nodes are one of the earlier groups of cells to be destroyed by x-ray radiation. These nodes show

complete regeneration within 21 days according to Bloom et al.[55] The splenic follicles, however, show complete regeneration in 14 days.[55] Moreover, it should be pointed out that not all of the reticuloendothelial elements are destroyed for such a long period of time, for 24 hours after radiation, the macrophages, reticulum cells, and epithelioid cells were still found undestroyed in the marrow, spleen, and lymph nodes.[55] Plasma cells are found within the lymph nodes 9 days after x-ray radiation. X-ray radiation destroys lymphocytes during the early phases of sensitization with horse serum in the rabbit, but even they begin to regenerate in the lymph nodes, spleen, and bone marrow towards the latter phase of the sensitization process. However, mature circulating lymphocytes in the peripheral blood stream are completely depressed during the entire phase of the sensitization process. Since the lymphocyte has been suggested as the source of antibody production and as a carrier of the circulating antibody, it might be assumed that the depression of the antibody titer may be attributed to the interference with the production of these cells. However, it cannot be assumed that the lymphocyte plays the entire role in antibody production. The plasma cells reappear within the lymph nodes 9 days after x-ray radiation and the phagocytes, epithelioid cells, and reticulum cells are present, undestroyed by x-ray within 24 hours after irradiation. Thus, it is entirely possible that these cells may be involved in the process of antibody production and, therefore, may contribute some antibodies to x-rayed, sensitized animals.

The above findings indicate that within the limits of this experiment, and taking into consideration the time element and the total x-ray dosage, there is evidence which shows that x-ray radiation depresses one part of the reticuloendothelial system, namely the lymphoid element. The fact that moderate to slight vascular and myocardial lesions were found, that serum precipitin titers were depressed but not negative in all instances, and that some of the Schultz-Dale reactions were positive even though all the Arthus phenomena were negative indicate that some part or parts of the reticuloendothelial organs other than the lymphoid tissues are not affected or completely destroyed by x-ray. These elements, then, may be the reticulum cells, the macrophage cells, or the epithelioid cells which are not destroyed by x-ray radiation, or the plasma cells which are depressed but return to the destroyed tissues in a very short time. Thus, since many parts of the reticuloendothelial system may play a role in antibody production, it may well be that when one tissue is destroyed within the reticuloendothelial system, other tissues or cells will partially take over its function. It also must be considered that antibody production of a lower quantitative and qualitative degree in these experiments may possibly have been carried out by regenerating lymphocytes during the latter phase of the sensitization process.

SUMMARY

1. A series of 40 adult rabbits were studied to reveal the effect of x-ray radiation on various phenomena of hypersensitiveness, namely, diffuse vascular disease and carditis, Arthus phenomenon, Schultz-Dale reactions, and precipitin formation.

2. Twenty-eight animals received 600 r and 800 r of x-ray radiation 24 to 72 hours before the sensitizing dose of horse serum. Four received no horse serum but received the x-ray radiation. Eight other animals were used as controls and received parallel doses of horse serum without x-ray radiation.

3. Microscopic sections of the irradiated and later sensitized animals showed a marked reduction in the number and intensity of vascular lesions and carditis, indicating the inhibitory action of x-ray on the development of these lesions. The Arthus phenomenon was consistently negative in the irradiated animals as compared with the controls. The Schultz-Dale reactions showed inconclusive and inconsistent results. The precipitin reactions showed depressed titers in the irradiated animals as compared to the controls.

4. Complete blood counts revealed marked leukopenia and depression of the lymphocyte counts on the irradiated animals as compared to the normal control animals.

5. X-ray radiation of normal nonsensitized animals showed no lesions of diffuse vascular disease or carditis.

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THE APHID—AN INSECT ALLERGEN*

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THE POTENTIAL of insects as inhalant allergens has been well established. Parlato[1-3] has shown that the emanations from the wing scales and hairs of the caddis fly were capable of producing hay fever and asthma in 5 per cent of his cases in the Lake Erie area. Parlato[4] and, later, Figley[5] concluded that hay fever and asthma were caused by the May fly in over 7 per cent of cases in the western Lake Erie section. Additional data[6] also include moths and butterflies in the list of inhalant allergens.

REASONS FOR THE INVESTIGATION

During the late summer and early fall of 1948 in New York City, Westchester County, and surrounding areas, there were an exceptional number of patients with asthma and coryza. While a number of these patients were ragweed problems, others were insensitive to this pollen by previous history and skin testing.

It has been observed for many years that many patients with summer and fall asthma and coryza fail to obtain relief from the usual pollen hyposensitizing therapy and continue with unabated symptoms during and beyond the period when grass and ragweed pollens are found in the air. While it is unquestionably true that some of these people are inadequately treated or are not responsive to treatment, there is also a likelihood that some hitherto unsuspected allergen is responsible for their discomfort.

During the fall of 1948 it was also noted that swarms of winged aphids were migrating in the Westchester area. These were so profuse that the following item appeared in the *New York Herald Tribune*[7]:

"The annual mating flight of aphids has brought such clouds of the tiny green insects to Westchester County that the residents are faced with the choice of being driven into their homes or out of their minds."

Believing these insects might in some fashion cause difficulty during the pollen seasons, an investigation into their life cycle was started. A number of the insects were caught and an extract was prepared. Skin testing with this extract produced definite positive reactions in many of the patients who were faring poorly on pollen therapy alone. Many of these patients' bloods were capable of producing positive passive transfer reactions. One patient had a systemic reaction from a skin test dose. All of these findings led to a continuation of the investigation.

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PERTINENT FACTS RELATING TO THE CHARACTER, DISTRIBUTION, AND LIFE CYCLE OF THE APHID

The aphid, or plant louse, is one of the most widely distributed members of the insect family. Geographically, it is found in all areas and in all climates in which vegetation is found. It is known to infest well over 7,000 food plants[8, 9] throughout the world and all of its food plants probably have not yet been catalogued.

Its economic significance is tremendous since the aphid destructively infests all varieties of cereals and grains, garden vegetables and flowers, fruit, hard wood and soft wood trees, in short, all manner of succulent vegetation including grasses, plantain, and weeds. The destructive green pea louse alone is estimated to have caused a loss of over $7,000,000 in two seasons just along the Atlantic Coast states.[10, 11]

An attempt to epitomize the life cycle of the aphid is like trying to draw an orderly sketch of chaos.[12] Simply stated, the aphid of the north, after having wintered as a gamogenetic (fertilized) egg upon its winter host, comes forth as a stem mother in the spring of the year. This stem mother reproduces a series of parthenogenetic (agamic) viviparous generations of female plant lice which feed upon the succulent spring foliage of their winter host. Later, because of a diminution of food supply, or by the dictates of reasons unknown, a generation of winged lice is formed which flies to the summer host of its species. Here, still by parthenogenetic viviparous reproduction, it reverts again to the wingless louse form and as such multiplies and feeds through the summer. With the onset of cold or unfavorable weather or with failure of its food supply, it again generates winged forms and flies to its winter host. These fall migrants, however, are bisexual and give rise to the gamogenetic egg which is the overwintering form of the insect. Actually, wide variations from the above life cycle take place, but are of little interest in a monograph of this nature. In the warmer climates, the Aphididae may retain their louse form and may continue parthenogenetic reproduction on a limited scale during periods of scant vegetative growth or they may hibernate during the period of their first instar. From this life cycle, it is obvious that the greatest numbers of aphids are present at the time of year the host is at its maximum vegetative vigor. Thus, different varieties of the aphids would be present in profuse numbers at different times of the year. The rosy apple aphis is at its numerical peak on the apple blossoms in late May and again on narrow and broad leaf plantain in September,[13] the potato aphid during August and early[14] September, etc. The forms taken during its life cycle are polymorphic. It is reproductively prolific. (Fig. 1.)

QUALIFICATIONS AS AN INHALANT ALLERGEN

The aphid is a most prolific insect. Eight to 10 days[15] following its birth, it begins its agamic reproduction. Each aphid will produce from 1 to 4 young daily for a total of 10 to 28 during its life. In the temperate zone there will be from 8 to 21 generations during a season. Allowing 10 young per generation and 10 generations a year 1 egg would produce 1,111,111,111 aphids were it not for enormous destruction by predators, disease, and unfavorable weather con-

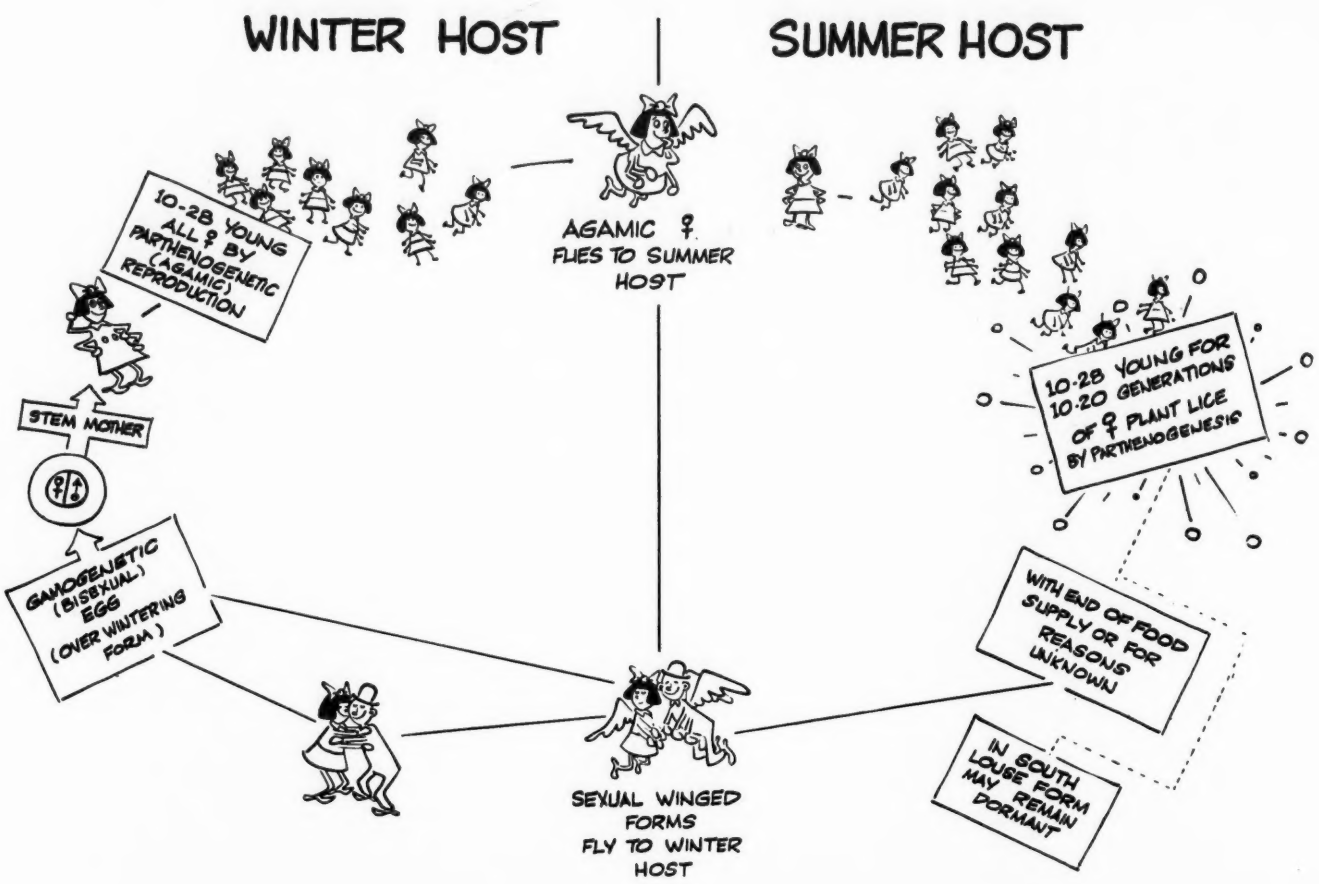
WINTER HOST
SUMMER HOST
10-28 YOUNG ALL ♀ BY PARTHENOGENETIC (AGAMIC) REPRODUCTION
AGAMIC ♀ FLIES TO SUMMER HOST
10-28 YOUNG FOR 10-20 GENERATIONS OF ♀ PLANT LICE BY PARTHENOGENESIS
STEM MOTHER
GAMOGENETIC (BISEXUAL) EGG (OVER WINTERING FORM)
WITH END OF FOOD SUPPLY OR FOR REASONS UNKNOWN
IN SOUTH LOUSE FORM MAY REMAIN DORMANT
SEXUAL WINGED FORMS FLY TO WINTER HOST

Fig. 1.—Cartoonist's version of the life cycle of the aphid. The gamogenetic egg develops to a stem mother who parthenogenetically reproduces female lice. These, in turn, parthenogenetically reproduce winged forms who fly to the summer host and revert to lice. These lice, all females, multiply prolifically, each producing 10 to 28 young for 10 to 20 generations during the summer. With the end of the food supply, sexual winged forms are developed who fly to the winter host and deposit the gamogenetic egg, the overwintering form. (Drawing courtesy Mr. James C. Raymond.)

ditions.[16] It has been estimated that the cabbage aphis[17] from one stem mother under perfect conditions would reproduce a sufficient quantity of progeny to cover the entire earth in one season.

Fig. 2.—Showing aphids and their cast skins upon a potato plant tip. The darker figures on the plant are the living lice and the lighter figures, the cast skins. (Courtesy Maine Agricultural Experiment Station, Orono, Me.)

During the course of its development from birth to adult form, the aphid progresses through 4 instars if it is to remain in the louse form and 5 instars if it is to become a winged form. At each instar it leaves a cast skin which is similar in type, although not in antigenic qualities, to that of the May fly.

Thus, the billions of aphids produce over four times their numbers of cast skins. These cast skins either remain for a period of time upon the food plant—being adhered by the action of honeydew—or fall upon the ground. It is logical to assume that these skins eventually dry and by breakage enter into the air as minute particles similar to the exuvia of the May fly. Because of the

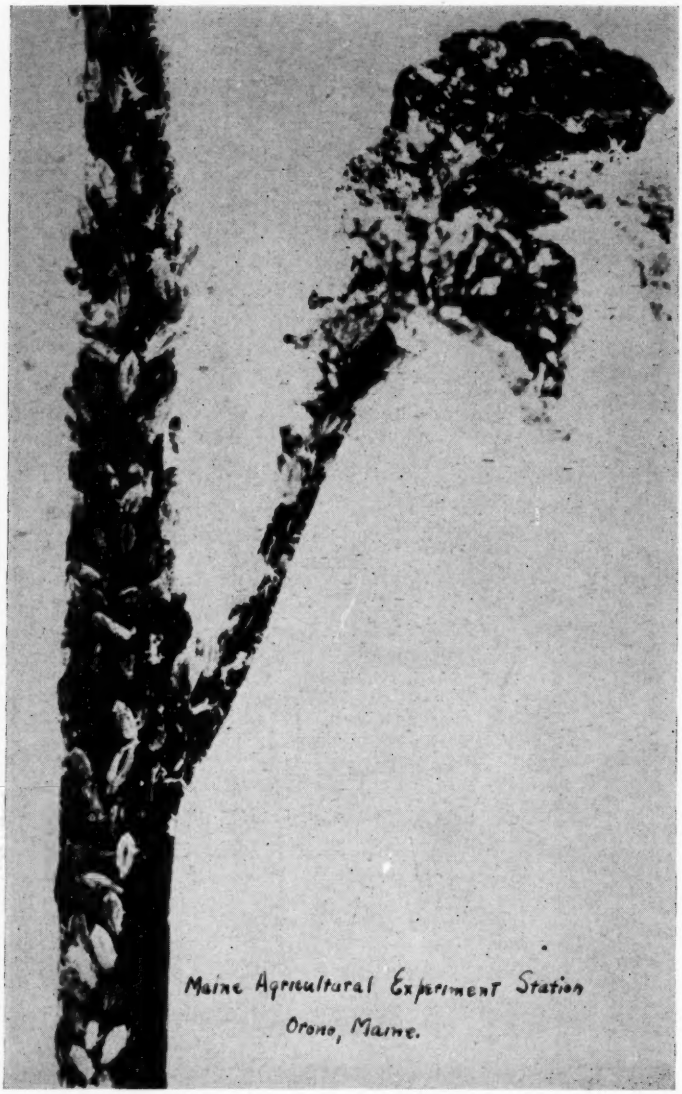

Fig. 3.—Enlargement of the central portion of Fig. 2. It amply demonstrates the dark living aphids, the white newly cast skins, and the small white shrunken cast skins.

abundance of the insects, it is also logical to assume that these exuvia are present in the air in not inconsiderable quantity. In our opinion, this material is the major aphid antigen, rather than the winged form of the insect which originally induced interest in this allergen.

Thus it is established that we have from the aphid a profuse quantity of light exuvia in minute particles. This is spread over a wide geographic area and is capable of contacting many allergic individuals. (Figs. 2 to 5.)

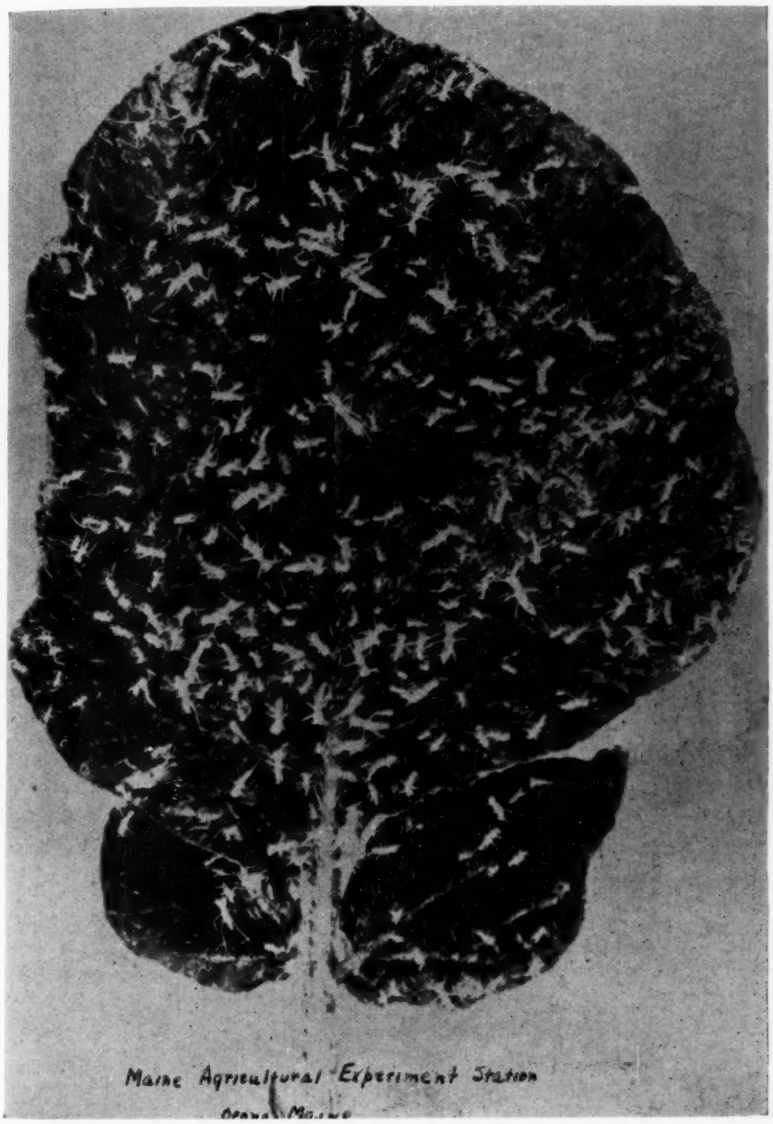

Fig. 4.—Photograph of a potato plant leaf, a large portion of which area is covered with the cast skins.

CHARACTER OF CAST SKINS (EXUVIA)

One of the anatomical characteristics of the insect is its exoskeleton. This is a relatively rigid structure giving the animal form as well as protection. During the process of growth the insect moults this structure at intervals.

Each ecdysis produces a cast skin. The number of moults or instars is constant for each species. The cast skins or exuvia is the discarded portion of exoskeleton. (Fig. 4.)

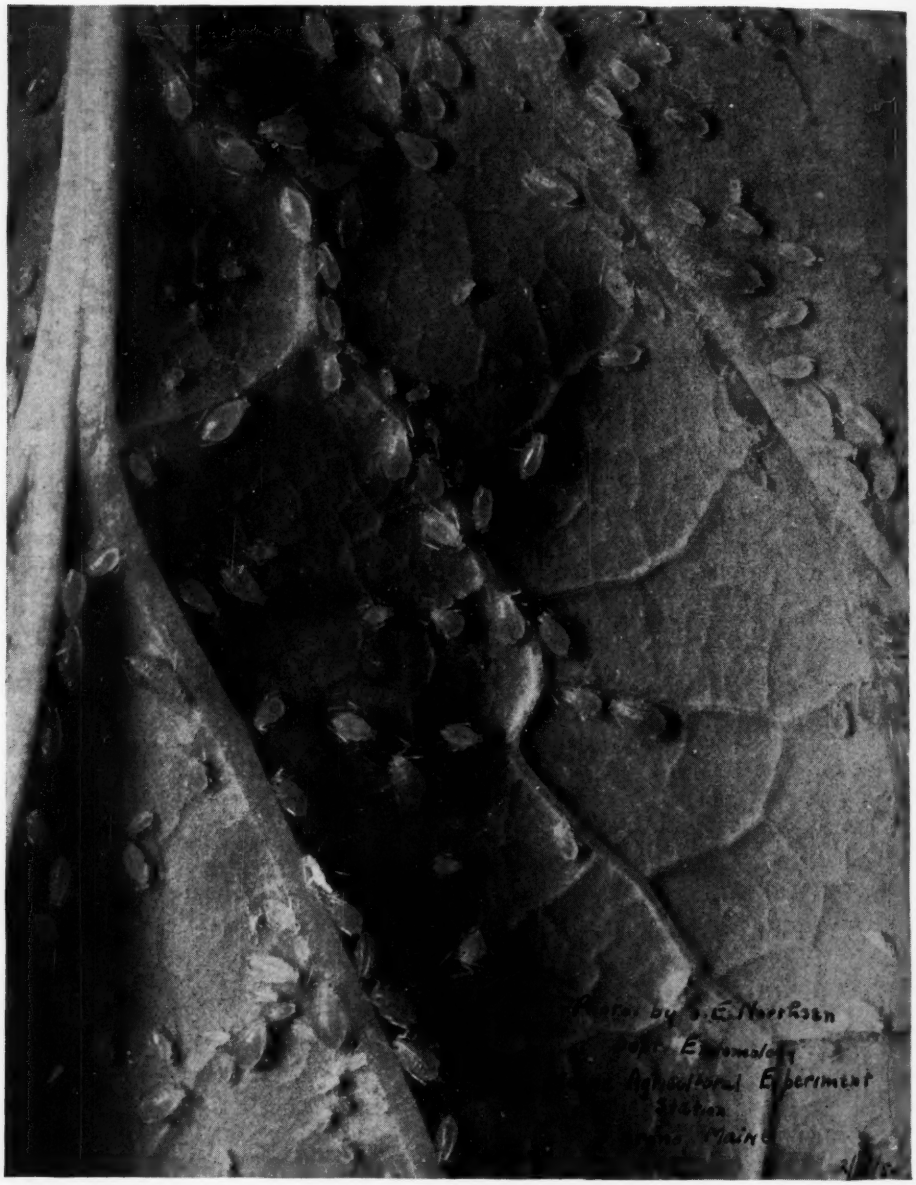

Fig. 5.—Close-up photograph showing the plant lice upon a peach tree leaf. There are 4 cast skins in the picture.

The exoskeleton or integument consists of cuticle. The cuticle[18] is a non-living material, the product of a living matrix. This material is a plasticized protein sheet with waxes externally and chitin internally.[19] Insect cuticle

contains chitin and a high proportion of other substances including pigment, fatty material, albuminous substance, and mineral components.[20]

Chitin is a nitrogenous polysaccharide, whose chemical composition is $C_{15}H_{26}O_{10}$. Of this, about 8 per cent is nitrogen, about one-half the nitrogen content of protein. The chitin of all insects is similar both chemically and by crystallography.[20] Chitin always occurs associated with protein. The proteins in the exoskeleton vary with each insect species.[20] The cuticle proteins are in some respects similar to silk, but are a different product. Since the proteins in the exoskeleton cuticle vary with insect species and since the proteins are probably the antigenic elements, we may be sure the insect antigens are species specific.

SOURCE OF MATERIAL

The subjects used in this investigation were all from private practice. The patients were under treatment for hay fever, nonseasonal coryza, and/or asthma. All had been thoroughly investigated medically and for hypersensitivities. Most of the cases were pollen sensitive, usually to ragweed.

The extract was prepared by utilizing all of the collected aphid material. Both winged and louse forms were present. The insects were defatted with toluene and extracted in Coca's solution. The extract thus obtained was dialyzed, filtered, and standardized by a total nitrogent determination. The total material available was 3 Gm., which was extracted in 100 ml. of Coca's solution. This formula produced an extract containing 0.16 mg. total nitrogen per ml. (T. N./ml.). The completed extract was clear and dark brown in color. The dilutions for skin testing were made with buffered saline.

METHOD OF INVESTIGATION

The patients were tested with aphid extract in the usual manner with the intracutaneous technique. Initial tests were performed with a testing strength of 0.001 mg. T. N. and 0.01 mg. T. N. After a number of patients had been tested, it was felt the higher dilution was unnecessary. This judgment proved correct in all but one case, in whom a systemic reaction occured from an injection of 0.01 mg. T. N. of the extract given intracutaneously. The volume of extract used for skin testing varied from 0.001 to 0.005 ml. The patients were or had been tested with ragweed, silk, and mould extracts in the strengths of 100 and/or 1000 Cooke-Stull protein nitrogen (P.N.) units. Silk was used as a testing material because of its possible antigenic relationship with other insect emanations. The moulds were tested routinely and were ruled out as probable allergens in most cases.

The patients selected were problem cases; they had what appeared to be typically allergic symptoms during the fall, both during and well beyond the period that ragweed pollen was found in the air, and they had received adequate pollen and/or inhalant therapy. Generally, these patients had received more than average attention since their treatment results were unsatisfactory.

The passive transfer tests were performed with the usual technique. Patients who had positive skin tests to the aphid and in whom the clinical story suggested sensitivity were bled. This blood was separated, and serum filtered,

TABLE I. RESULTS OF DIRECT SKIN (INTRACUTANEOUS) TESTS

CASE NUMBER	APHID 0.01 T.N. UNITS PER C.C.	ALTERNARIA 1000 P.N. UNITS	RAGWEED 1000 P.N. UNITS	SILK 1000 P.N. UNITS
1	neg	mkd	neg	neg
2	neg	neg	neg	mkd
3	neg	mod	mod	sl
4	neg	mkd	slmod	slmod
5	neg	neg	sl	slmod
6	neg	mkd	mkd	mkd
7	mod	mod	mkd	mod
8	neg	slmod	mkd	modmkd
9	modmkd	mkd	mod	mod
10	neg	mkd	mkd	mod
11	neg	mkd	neg	mkd
12	mod	mod	neg	mod
13	slmod	mod	mkd	slmod
14	neg	mkd	mkd	mod
15	slmod	sl	mkd	slmod
16	mkd	mod	mod	mkd
17	neg	neg	neg	neg
18	neg	neg	neg	neg
19	neg	neg	mod	neg
20	neg	mkd	sl	mkd
21	neg	mkd	mkd (a)	mod
22	neg	sl	mkd (a)	mkd
23	sl	mkd	mkd (a)	sl
24	neg	neg	sl	neg
25	sl	mkd	mkd	mod
26	mkd	---	neg	neg
27	mod	---	mkd	---
28	mod	---	mkd	---
29	neg	sl	mkd	neg
30	neg	neg	neg	neg
31	mkd	neg	neg	mkd
32	mod	neg	neg	neg
33	mkd	neg	mkd	sl
34	mkd	sl	mkd	mod
35	neg	neg	mkd	neg
36	sl	mkd	mkd	mkd
37	neg	mod	mkd	neg
38	mkd	sl	mkd	neg
39	sl	neg	mkd	neg
40	mod	neg	mkd	neg
41	mod	mkd	mod	sl
42	mod	neg	mkd	mod
43	neg	neg	mkd	neg
44	mkd	sl	mkd	neg
45	mod	neg	mod	neg
46	neg	neg	mkd	sl
47	mkd	neg	mkd	mkd
48	neg	neg	neg	neg
49	mkd	---	mkd	---
50	sl	mod	mod	neg
51	mkd	mod	mkd	mkd
52	mkd	neg	neg	neg
53	mkd	mkd	mkd	neg
54	mkd	neg	mod	sl
55	mkd	mkd	mkd	mod
56	neg	mod	mkd	mkd
57	mod	neg	mkd	sl
58	sl	neg	neg	neg
59	mod	neg	mod	mod
60	mkd	mkd	mod	slmod
61	mod	neg	neg	neg
62	mkd (a)	mkd	mkd	neg
63	mkd	neg	sl	mod

TABLE I—CONT'D

CASE NUMBER	APHID 0.01 T.N. UNITS PER C.C.	ALTERNARIA 1000 P.N. UNITS	RAGWEED 1000 P.N. UNITS	SILK 1000 P.N. UNITS
64	mkd	mkd	mod	slmod
65	modmkd	neg	mod	neg
66	mkd	mod	mkd (a)	neg
67	neg	neg	mod	neg
68	neg	neg	neg	neg
69	mkd	neg	neg	mod
70	mod	sl	mkd (a)	mod
71	mkd	mod	mod	mkd
72	neg	mkd (a)	mkd	neg
73	mkd	neg	mkd	neg
74	mod	mod	mkd	mod
75	mkd	neg	mod	neg
76	mod	neg	mkd	sl
77	mod	neg	mkd	neg
78	sl	mkd	mkd (a)	sl
79	neg	neg	mod	neg
80	mkd	mkd	mkd	neg
81	mkd (a)	mkd	mod	mod
82	neg	mod	mod	mod
83	slmod	neg	neg	mod
84	slmod	mod	mkd	neg
85	neg	mod	mkd	mod
86	mkd	sl	neg	mkd
87	mkd	neg	mkd	neg
88	mod	mkd	mkd	mkd
89	mkd	mod	mkd	mod
90	neg	mod	mkd	mkd
91	mod	mkd	mkd	neg
92	slmod	mkd (a)	mkd	neg
93	mkd	mod	mod	mod
94	neg	sl	mkd	neg
95	neg	mod	neg	neg
96	mkd (a)		mkd	mod
97	mkd		sl	mod
98	mod		mkd	sl
99	mod		mod	mod
100	(systemic reaction) mkd aphid 0.001 mod		neg	mkd
101	mkd		mod	modmkd
102	mod		modmkd	sl
103	mkd		mkd	neg
104	mkd		mod	neg
105	mkd		mkd	sl
106	mkd		mod	sl
107	mkd		mod	neg
108	modmkd		mkd	mod
109	mod		sl	neg
110	slmod		slmod	neg
111	mod		mkd (a)	sl
112	modmkd		mkd	mod
113	mkd		mkd	mkd
114	mkd		mkd	sl
115	mkd		mkd	
116	mkd		modmkd	mod
117	modmkd		mkd	modmkd
118	mkd		modmkd	sl
119	mkd		mkd	mod
120	mod		neg	neg
121	mkd		mkd	sl

All cases had asthma and/or coryza with symptoms aggravated during the summer and fall.

Units: Cooke-Stull protein nitrogen.

Key:

neg	negative
slmod	slight to moderate
mod	moderate
modmkd	moderate to marked
mkd	marked
mkd (a)	marked active

and 0.1 c.c. of the sterile serum was injected in selected sites into the skin of the donor. These prepared transfer sites were tested at the end of 24 hours and were exhausted as rapidly as possible. Following exhaustion of the sites with either ragweed or aphid, retests were performed with the alternate material.

The initial investigation was performed on patients in whom the symptoms were related to a large degree to the ragweed season. For this reason, it was felt it would be well to prove an absence of antigenic relationship between this pollen and the aphid. To impress this fact, transfer sites were tested and exhausted with aphid or ragweed; then retested with ragweed or aphid. (Tables II and III.)

The ophthalmic tests were performed upon only a few patients by placing a drop of the extract in the conjunctival sac. These tests were read within 15 minutes. The characteristic conjunctival vascular dilitation was interpreted as a positive reaction.

RESULTS

In the falls of 1948 and 1949, a total of 121 patients were tested with the aphid extract. Of these, 75 gave reactions interpreted as positive. The results of these tests and of tests in the same patients with ragweed, alternaria, and silk may be seen in Table I.

Among the 75 who gave positive reactions, 59 had clinical symptoms which were worse in the summertime, usually much worse in the fall, and the remainder had symptoms of equal intensity throughout the year. Most patients were prone to have difficulty during the so-called pollen-free period from mid-July until mid-August and were especially bad during September and October. In many cases evidence of upper respiratory infection was evident. This, however, was not greater than might be found in the same patient at other times of the year with milder manifestations of coryza and asthma, or with none at all. One patient, following an intracutaneous test dose, had a severe systemic reaction, which occurred within 10 minutes of the test. This consisted of angioneurotic edema of the face and eyes and asthma. Epinephrine was necessary to control the reaction.

The remaining 46 cases failed to give positive aphid skin tests. The possible reasons for this are too obvious for discussion.

Case 96, in whom a marked active aphid test was elicited, was extremely sensitive to yellow jacket. On one occasion following a sting from this insect, the patient became unconscious, asthmatic, and cyanotic with evidence of edema of the larynx. This may account in part for the activity of the aphid test. The patient, however, also is a ragweed, fall, and winter asthmatic.

Several of these cases were able to obtain some relief of their symptoms by residence in New York City during September and October. It is interesting to note that the ragweed pollen count in New York City has consistently been higher than in the White Plains area. This apparent phenomenon is readily explained. The Westchester area is, to a large extent, well tended and the unpopulated areas are well covered with perennial vegetation, leaving little area for ragweed. The many open lots in the boroughs surrounding Man-

hattan and in New Jersey lead to the propagation of large areas of ragweed from which pollen is carried to the city, regardless of the wind direction. The aphid, on the other hand, finds vegetation in more profuse quantity in the suburbs; hence, it is present in greater numbers. This may explain the symptomatic relief obtained in some patients by metropolitan residence.

The figures in Table I reveal, also, that 44 cases gave positive skin reactions to alternaria, 90 to ragweed, and 46 to silk. Among the 75 cases positive to aphid, 41 were negative to silk. Among the 46 cases positive to silk, 19 were negative to aphid. These figures would indicate an absence of antigenic relationship between the two antigens. Among the 75 cases positive to aphid, 14 were negative to ragweed. Among the 90 cases positive to ragweed, 31 were negative to aphid. These figures would indicate a probable absence of relationship between these two antigens. This was substantiated by the passive transfer tests. (Tables II and III.) Among the 75 cases positive to aphid, 29 were negative to alternaria. Among the 44 cases positive to alternaria, 21 were negative to aphid. Here again, an absence of antigenic relationship is probable. All of this data would indicate an absence of common antigenic qualities among the four materials tested.

TABLE II. RESULTS OF PASSIVE TRANSFER TESTS

CASE NUMBER	RESULT OF DIRECT SKIN TEST WITH APHID 0.01 T.N. UNITS PER C.C.	PASSIVE TRANSFER TEST APHID 0.1 T.N. UNITS PER C.C.		RAGWEED 2000 P.N. UNITS
1	mkd	mkd	s.e.a.	mod
2	mkd	modmkd	s.e.a.	sl
3	mkd	mod	s.e.a.	mod
4	mkd	mkd (a)	s.e.a.	sl
5	mkd	mkd	s.e.a.	mkd
6	mkd	mod	s.e.a.	sl
7	mkd	mkd	s.e.a.	mkd
8	mkd	mod	s.e.a.	sl
9	mkd	mkd	s.e.a.	sl
10	modmkd	mod	s.e.a.	mkd
11	mkd	mod	s.e.a.	neg
12	mkd	mod	s.e.a.	sl
13	mkd	mod	s.e.a.	neg
14	modmkd	slmod	s.e.a.	mod
15	mkd	mod	s.e.a.	sl
16	mkd	slmod	s.e.a.	mkd
17	mkd	mod	s.e.a.	mkd

s.e.a. - site exhausted to aphid.
Each test was controlled.
All controls were negative.
For key to table, see footnote to Table I.

In general, it may be logically deduced that such a large number of positive skin reactions to aphid extract in the cases described above would indicate the possibility of sensitivity to this material. (Table I.)

The results of the passive transfer tests may be found in Tables II and III. Of 17 bloods tested, 15 gave positive transfer reactions to aphid extract; 6 of these reactions were marked. In this group of 17 cases, 14 were clinically sensitive to ragweed. Of the 14 ragweed cases tested, 12 gave positive transfer reactions to the ragweed.

TABLE III. RESULTS OF PASSIVE TRANSFER TESTS

CASE NUMBER	RESULT OF DIRECT SKIN TEST IN RAGWEED	PASSIVE TRANSFER TEST RAGWEED 2000 P.N. UNITS		APHID 0.1 T.N. UNITS PER C.C.
1	mod	mod	s.e.r.	mod
2	mkd	mod	s.e.r.	mod
3	mkd	mod	s.e.r.	sl
4	mkd	mod	s.e.r.	mkd
5	mkd	mkd	s.e.r.	mod
6	mkd	sl	s.e.r.	sl
7	mkd	mkd	s.e.r.	mod
8	mod	mod	s.e.r.	mod
9	mod	mod	s.e.r.	mod
10	mkd	mkd	s.e.r.	sl
11	modmkd	neg	s.e.r.	sl
12	mod	neg	s.e.r.	mod
13	neg	neg	s.e.r.	mod
14	mod	slmod	s.e.r.	sl
15	mod	neg	s.e.r.	mod
16	mkd	mkd	s.e.r.	sl
17	mkd	mkd	s.e.r.	slmod

s.e.r. - site exhausted to ragweed.
All controls were negative.
For key to table, see footnote to Table I.

All of the passive transfer test sites were exhausted, then retested with the alternate material. Of the 15 cases who had successfully transferred with the aphid extract, 10 still had skin-sensitizing antibody to the aphid after site exhaustion by ragweed. (Table I.) Of the 12 cases who had successfully transferred with ragweed extract, 8 were capable of demonstrating skin-sensitizing antibody to ragweed after site exhaustion with aphid.

These results would indicate that the patients with positive skin tests to the aphid extract have a skin-sensitizing antibody. They would indicate, also, that the aphid antigen is in no manner related to ragweed. (Table II and III.)

This work is presented as a preliminary report, with the hope of stimulating other workers. Much remains to be done to further prove the validity of these contentions. Efforts now in progress should produce some interesting data within the next few months.

SUMMARY

1. The life cycle of the aphid is explained. The aphid is one of the most prolific insects known.

2. An explanation of the qualifications of the aphid as an air-borne inhalant allergen is offered.

3. A number of patients with seasonal allergic manifestations have symptoms which are not explained by the known seasonal allergens.

4. Seventy-five of 121 patients, whose symptoms were unexplained by known seasonal inhalant allergens, gave positive reactions to aphid extract.

5. One patient had a systemic reaction of marked intensity following a skin test with aphid extract.

6. Passive transfer tests indicate that transferable skin-sensitizing antibody is present in the blood of skin-sensitive individuals and that the antibody is not antigenically related to ragweed.

7. It is felt that the aphid is an important insect inhalant allergen heretofore unnoticed.

Acknowledgment is given to Mr. Schuyler Purdy for his technical assistance, and to Drs. Geddes W. Simpson, F. H. Lathrop, and Donald Folsom of the University of Maine Agricultural Experiment Station for their interest, information, and advice on the entomological data and for their continuing efforts in this direction.

The cooperation of Dr. Albert Hartzell of the Boyce Thompson Institute is greatly appreciated.

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SKIN SENSITIVITY TO STREPTOMYCIN. III*†‡

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IN ONE earlier paper,[1] we discussed the cause, symptoms, diagnosis, and treatment of skin sensitivity to streptomycin. A later one[2] elaborated on dry patch tests for sensitivity to both streptomycin and dihydrostreptomycin (for which the abbreviation DHS is used). The present study is later and more detailed and includes data from several nurses studied earlier with some new cases added.

Ninety-one nurses were tested. (Table I.) Only 3 had never handled streptomycin or DHS. All 3 of these controls were negative to streptomycin and DHS patches. One had been tested in the earlier series and was also negative at that time.

Twenty-eight nurses had handled streptomycin but not DHS. None of them had ever had any eruption from streptomycin nor had ever been given any of the drug therapeutically. Eighteen had been tested previously and were negative. Streptomycin had been handled from 1 week to 4 years and the average for the whole group was 16 months. Practically all of the nurses

Table I. Contact With Streptomycin

NO. OF NURSES	HANDLED DHS	TIME STREPTOMYCIN HANDLED—RANGE	AVERAGE TIME STREPTOMYCIN HANDLED	HOW OFTEN HANDLED STREPTOMYCIN—RANGE	LAST TIME STREPTOMYCIN HANDLED—RANGE
28	No	1 week to 4 years	16 months	daily to monthly	same day to 1 year ago
63	Yes	1 week to 4 years	18 months	daily to monthly	same day to 1 year ago

had handled the drug several times daily but 5 had contact with it less often. The time that had elapsed since handling streptomycin varied from the same day to one year previously. One-half of the nurses had contact within 2 weeks of the test and most of these the same day. All were negative to streptomycin and DHS dry patch tests.

The remainder of the nurses tested (63) had handled both streptomycin and DHS. Contact with the former averaged 18 months and ranged from 1 week to 4 years. In frequency, also, there was little difference, being from several times daily to once a month or less. The elapsed time also was from the same day to one year previously with over one-half having had contact within a day of the test. (Table II.) Twenty-eight had been tested one year ago, were negative then, and are still negative. Twenty-six had not been tested previously but were negative at this time.

*From the Allergy Clinic, Brown General Hospital, Veterans Administration Center, Dayton, Ohio.

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‡Read before the Sixth Annual Meeting of the American Academy of Allergy, Los Angeles, Calif., March 6-8, 1950.

Of the remaining 9 nurses, we had tested 6 previously. Four had been positive then and were positive now. One (M) who had been negative one year ago was now positive and one (J) who had been positive a year ago was now negative (no contact with either streptomycin or DHS for 5 months previous to test). Two nurses (Z) had not been tested before. One gave a positive and one a negative reaction. This second nurse is included in this group because as long as the patch remained in contact with her skin she complained of swollen and itching eyes. These symptoms also occurred on handling either streptomycin or DHS but she had never had a skin eruption. The other nurse in this group (N) first developed a rash in 1947 and received elsewhere an intradermal test to streptomycin. This was read at that time as positive and was followed by a generalized urticaria which lasted 3 months. An evanescent papular eruption was produced by the patch test.

TABLE II. COMPARISON OF PREVIOUS DRY PATCH TESTS FOR STREPTOMYCIN AND DHS SENSITIVITY AND THE PRESENT ONES

INITIAL	NO. OF NURSES	HANDLED STREPTOMYCIN	HANDLED DHS	PREVIOUS TEST		PRESENT TEST	
				STREPTOMYCIN	DHS	STREPTOMYCIN	DHS
	3	no	no	neg. (1)	neg. (1)	neg.	neg.
	28	yes	no	neg. (18)	neg. (18)	neg.	neg.
	54	yes	yes	neg. (28)	neg. (28)	neg. (54)	neg. (54)
Q	4	yes	yes	pos.	pos.	pos.	pos. (2)
M	1	yes	yes	neg.	neg.	pos.	neg.
J	1	yes	yes	pos.	neg.	neg.	neg.
Z	2	yes	yes	none	none	pos. (1)	pos. (1)
N	1	yes	yes	intradermal pos.	none	pos.	pos.

Three nurses had been treated therapeutically with streptomycin, 2 a year before and the other 5 months before test. Two had handled DHS and streptomycin quite frequently. None of these had developed any contact sensitivity and all were negative to streptomycin and DHS dry patches.

Two nurses who had extensive contact with streptomycin and DHS without developing any eruption had previously developed skin reactions to injected penicillin. One had a contact sensitivity to penicillin the other none. Streptomycin and DHS dry patch tests were negative. (Table III.)

TABLE III. CONTACT WITH DHS

NO. OF NURSES	HANDLED STREPTOMYCIN	TIME DHS HANDLED—RANGE	AVERAGE TIME DHS HANDLED	HOW OFTEN HANDLED DHS—RANGE	LAST TIME DHS HANDLED—RANGE
63	yes	1 day to 10 months	5½ months	daily to every 2 weeks	same day to 8 months ago

Of the 63 nurses who had handled DHS, the contact has existed from one day to 10 months with the average for the group 5½ months. Contacts varied from several times each day to about once every 2 weeks, 49 of the 63 nurses

handling the drug daily. The last contact before test was from the same day to 8 months before, with 29 having handled the drug the day of the test. (Table II.)

Four nurses gave positive reactions to the dry DHS patches. All of these were also positive to the streptomycin dry patches. Two had been tested 9 months previously and were also positive. The other 2 had not been tested before. Only one of the 4 had developed an eruption although all had had daily contact with streptomycin and DHS, streptomycin from 14 months to 3 years and the DHS from 4 months to 10 months.

One nurse (M) who had a positive dry patch to streptomycin was negative to DHS but had only handled it for one week 2 months previous to the tests. Two other nurses (Q), now positive to dry streptomycin patches and who had positive reactions to dry DHS 9 months ago, were now negative to dry DHS patches. Both of these had a great deal of respect for the drugs and only handled them or syringes containing them with rubber gloves. Why there had been a change in the DHS sensitivity and not in the streptomycin sensitivity is not known although the streptomycin sensitivity was the oldest and was developed before any DHS was used in the hospital. One of these also has a contact sensitivity to penicillin.

Eight of the 91 nurses admitted to having developed a skin eruption since handling streptomycin. Four of these were negative to dry patch test and on investigation were believed to have other contact sensitivities. The other 4 (Q) were positive to dry streptomycin patches and all are either now positive to dry DHS patches, or were 9 months ago.

These eruptions lasted from 2 days to 5 months, the average being about 2 weeks. Treatment consisted of any or combinations of the following: antihistamine tablets, ointment, or cream, Calomine lotion, epinephrine, ultraviolet lamp, or no treatment.

DISCUSSION

With more contact with DHS, the points elaborated in an earlier paper[2] seem to be borne out. Without contact, no sensitivity develops. Skin sensitivity to streptomycin does not mean there will also be a sensitivity to DHS unless the latter is frequently handled. So far, we have found no contact sensitivity to DHS without contact sensitivity to streptomycin but none of our nurses have handled only DHS. Apparently, skin sensitivity to DHS and streptomycin may be lost, probably temporarily, by avoidance of contact with them. At any rate, the precautions to be taken by those handling the drugs to prevent the development of sensitivity might possibly cause this sensitivity to disappear if these rules are religiously followed. Otherwise, there seems to be no better treatment of the skin eruption at the present time than antihistamine in tablets orally, and by local application in ointments or creams. We routinely test these patients to molds and hyposensitize to any to which they show positive immediate or delayed skin reactions even though they be the equivocal Trichophyton or oidiomycin. The nurses so treated have felt that their resistance

to further eruption is somewhat increased. Even with the best intentions, occasional contact cannot be prevented unless another type of work is chosen.

It is again reiterated that these tests should be done routinely on personnel handling the drugs, probably every 6 months. The rules for prophylaxis given previously should be rigidly carried out although we are forced to admit that certain individuals, regardless of contact, will probably never develop a skin sensitivity.

Certain very sensitive individuals have noted exacerbation of rash or swelling of the eyelids while the patch is in contact with the skin. We make a practice in such patients of leaving the patch on but 24 hours instead of the usual 48.

As far as percentage of sensitization is concerned, 7.7 per cent of those handling streptomycin became sensitized while 6.3 per cent of those handling DHS became sensitive.

The present series has demonstrated also that the dry patches do not lose in effectiveness on being stored at room temperature for a year.

SUMMARY

1. Contact sensitivity to streptomycin and DHS may develop in those frequently handling the drugs if certain precautions are not taken.

2. Certain individuals do not become sensitized and others may lose their sensitivity by avoidance of contact.

3. The best treatment of the eruption, at present, seems to be with antihistamine drugs.

4. The dry test patches used are still effective after storage at room temperature for one year.

The author would like to express his appreciation and thanks to Dr. Ray C. Pogge of Merck and Company, Inc. for his help in the preparation of the patches, and to Mrs. Lila Rinard, B.S., for technical assistance.

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TESTS OF DIFFERENT MEDIA FOR THE COLLECTION AND IDENTIFICATION OF AIR-BORNE SAPROPHYTIC FUNGI*

MARY ANN SWAEBLY, PH.D., CLYDE M. CHRISTENSEN, PH.D., AND THEODORE A. GRAHEK, B.S., ST. PAUL, MINN.

THE purpose of the extensive work with media favorable for the collection and identification of air-borne fungi recently done at the University of Minnesota, Division of Plant Pathology and Botany, in cooperation with the American Academy of Allergy, was to determine whether the casein hydrolysate medium being used in the present air-borne mold survey is giving the highest possible total mold count or whether other media could be found which would give significantly higher counts.

The test method used in all of the experiments was essentially the same as that recommended by Dr. Leonard Harris in his mimeographed letter to the members of the Academy of Allergy who are cooperating in the mold survey.

In preliminary tests, 2 plates of each of 18 different media were exposed for 12 minutes, the plates being arranged in a randomized block. A new set of plates were exposed on each of 3 successive days. Those media which gave consistently low total mold counts, or which obviously supported the growth of fewer genera than the other media in the series, were eliminated from further tests. Fifteen media were eliminated in the preliminary tests. The 3 remaining media were then used in all of the exposures from June 1 to September 15. The 3 which proved satisfactory in the preliminary tests and which were tested further were: (1) modified Smith-Humfeld medium plus sodium chloride; (2) Waksman's medium plus sodium chloride; and (3) Mehrlich's medium plus sodium chloride.†

The casein hydrolysate medium‡ was used as a standard against which to compare all results throughout the experiment except in the preliminary screening, and was included in all of the exposures.

*Read before the Sixth Annual Meeting of the American Academy of Allergy, Los Angeles, Calif., March 6-8, 1950.

†The formulas for the media used in our comparative tests are as follows:

1. Modified Smith-Humfeld plus sodium chloride:

KH_2PO_4	1Gm.
$NaNO_3$	1Gm.
Dextrose	15Gm.
NaCl	75Gm.
Agar	15Gm.
Distilled H_2O	1 liter

2. Waksman's medium plus sodium chloride:

K_2HPO_4	1Gm.
$MgSO_4$	0.5Gm.
Peptone	5Gm.
Glucose	10Gm.
NaCl	75Gm.
Agar	15Gm.
Distilled H_2O	1 liter

3. Mehrlich's medium plus sodium chloride:

KH_2PO_4	1Gm.
$MgSO_4$	0.5Gm.
Peptone	5Gm.
Malt extract	5Gm.
Dextrose	15Gm.
NaCl	75Gm.
Agar	15Gm.
Distilled H_2O	1 liter

‡Casein hydrolysate plus sodium chloride (prepared Mead Johnson & Company laboratory product):

Casein hydrolysate	50Gm.
NaCl	75Gm.
Agar	15Gm.
Distilled H_2O	1 liter

Each medium was judged on the following 4 points considered important in the selection of a substratum for the collection and identification of fungi: (1) The number of identifiable colonies obtained; (2) the number of genera obtained; (3) restricted colony size to eliminate "masking" effect and coalescing of colonies, and (4) facility in identification of the fungus colonies.

Preliminary tests indicated that approximately one mole of NaCl added to any medium greatly increased its usefulness for mold survey work, by practically eliminating bacterial contaminants and by restricting colony size, thus reducing the tendency for the colonies to coalesce. Therefore 75 Gm. of NaCl were added to each liter of medium (Fig. 1).

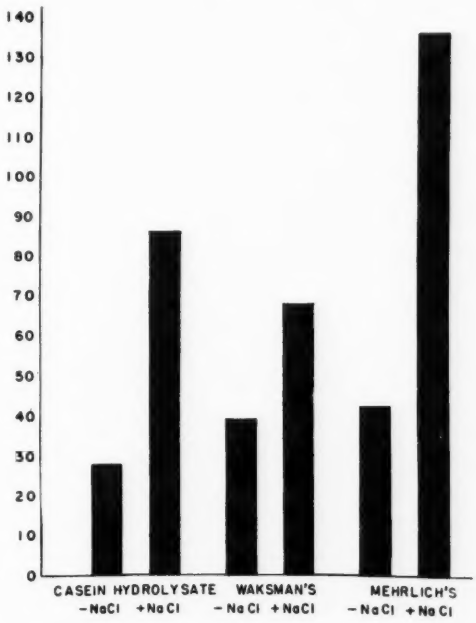

Fig. 1.—Average number of colonies per plate on 3 media with and without sodium chloride.

There were no significant differences in the number of genera obtained on any of the 4 media, except in the case of the modified Smith-Humfeld salt agar which would not support the growth of many of the Phycomycetes, especially Rhizopus and Mucor, but there were considerable differences in number of colonies, and in relative prevalence of some of the common air-borne molds.

The casein hydrolysate medium which is now used in the survey does not compare favorably with several of the other media, even after the addition of the salt. This medium gave the second lowest total mold count of those tested extensively. It permits a profuse growth of many fungi, but this abundant growth was partly responsible for the low total count, since the colonies merged into each other, leaving no sharply defined borders; consequently the colonies failed to stand out as individuals. For example, when one of the Aspergilli or Penicillia predominated, 2 or 3 colonies would merge into each other, and, unless they were counted with the aid of a microscope before they were clearly

visible to the unaided eye, several colonies could easily be mistaken for one colony. Similarly, if Rhizopus or Mucor were present, a plate could be completely covered with dense mycelium in 24 to 48 hours and if other fungi were present underneath the dense overgrowth, they could neither be counted nor identified (Fig. 2).

From the standpoint of total mold counts and facility in identification, a modified Smith-Humfeld salt agar was far superior to all others, but because Rhizopus and Mucor do not grow well on it, its use for survey purposes is perhaps limited. The extremely high average colony count per plate on this medium was due, mainly, to the large number of Hormodendrum and Alternaria colonies which appeared during the early summer weeks. (Figs. 3 and 4). On this agar, it was possible to get fairly accurate counts of the extraordinarily large number of colonies for two reasons; first, the colonies remained small, and second, the dark reverse side of the sharply defined Hormodendrum colonies was clearly visible through the almost transparent agar.

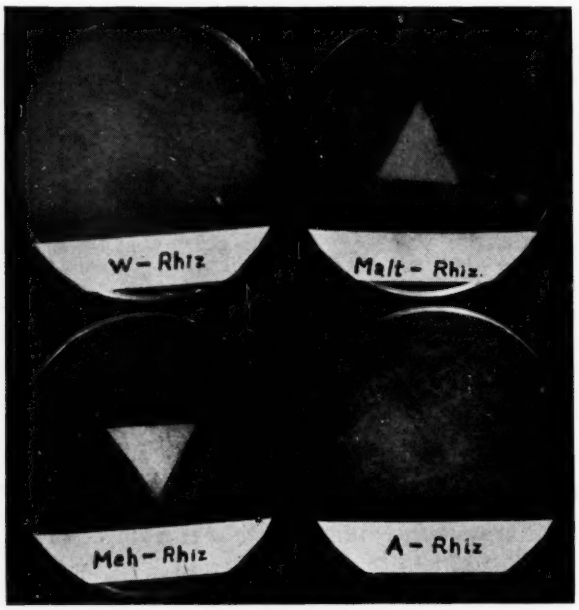

Fig. 2.—Cultures of Rhizopus growing on 4 media. *W.* Waksman's; *Meh.* Mehrlich; *A*, casein hydrolysate. White paper triangles beneath the dishes are not visible through the dense mycelium on Waksman's agar and on casein hydrolysate.

Attempts are now being made to modify this agar further so that common air-borne Phycomycetes will grow upon it. If this can be done, this agar should prove to be extremely useful in survey work.

During the first weeks of the experiment Waksman's agar plus salt appeared to be an excellent one for the collection and identification of air-borne fungi because of the adequate but limited growth of the colonies. When Alternaria and Hormodendrum became prevalent, however, the total count on Waksman's medium failed to rise as sharply as did the total count on the other media. Comparison of the daily figures indicated that this medium consistently

was not permitting the growth of as many colonies as the other media, consequently it was eliminated from the series.

In our tests the most favorable medium was Mehrlich's agar plus sodium chloride. It is light tan in color, fairly transparent, and supports the growth of many common air-borne saprophytic fungi predominant in this region. Its chief advantages are that the colonies remain small and sharply delimited, hence they are clearly visible at maturity as separate entities, eliminating the necessity for observing the plates microscopically before the colonies are mature. After a little practice it is not difficult to identify the fungi by gross cultural observations on this medium, although most fungi appear generally somewhat darker and "dusky" without the bright colors characteristic of many colonies on the casein hydrolysate medium. In our tests Mehrlich's medium gave a high mold count, second only to the modified Smith-Humfeld salt agar, which, as mentioned before, is limited in its usefulness because of its inability to support the Phycomycetes.

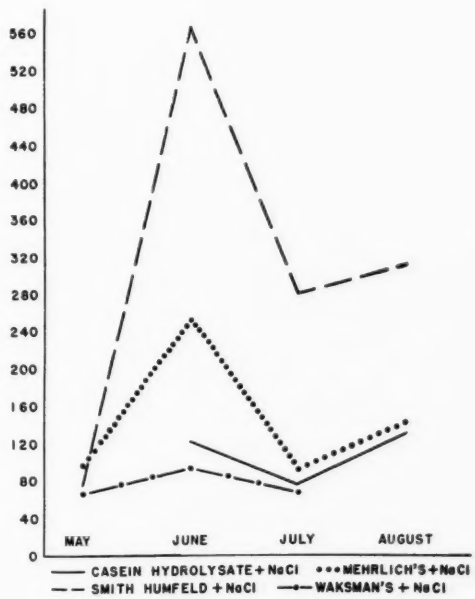

Fig. 3.—Average number of colonies per plate for 4 months on the 4 most favorable media.

Growth of all fungi was adequate for identification, but at the same time the colonies were greatly reduced in size over similar colonies grown on the casein hydrolysate medium, and the border of each colony was sharply defined. Rhizopus and Mucor grew sufficiently for easy identification, but not densely enough to obscure the other colonies on the plates (Fig. 2).

In the preliminary experiments, a malt salt agar gave very high mold counts, but the fungi were often difficult to identify because of the very dark color of the agar when prepared with a crude grade of malt extract. During the last few weeks of the experiment, however, a highly purified malt extract was obtained and the resulting agar was transparent as well as much lighter in color.

As a result the typical color and texture characteristics of the individual fungi were clearly evident on gross examination, although the same "duskiness" described on the Mehrlich's medium persisted here. Since, however, this form of the malt agar has not been tested extensively under actual survey conditions, we cannot at this time make any definite statement regarding its value for this type of research. It appears to have considerable promise.

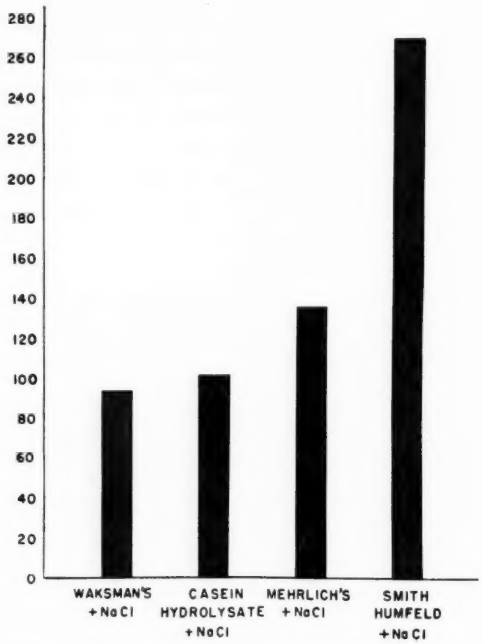

Fig. 4.—Total average number of colonies per plate for 4 months, May, June, July, and August, on the 4 most favorable media.

SUMMARY

1. The experimental results to date indicate that Mehrlich's medium with 75 Gm. of sodium chloride per 1000 c.c. of H_2O will give a significantly higher mold count per plate than the casein hydrolysate medium now employed in the air-borne mold survey. This medium supports the growth of a large number of the air-borne saprophytic fungi common to this region.

2. There is evidence, however, that this medium is still not the best possible, since readings from the modified Smith-Humfeld salt agar show a mold count almost double the mold count obtained on Mehrlich's salt agar and almost triple the mold count from the casein hydrolysate salt medium.

3. As a result, therefore, experiments with a further modified Smith-Humfeld salt medium, a purified malt salt agar, and reduced strengths of the casein hydrolysate medium are being continued.

4. None of these media will disclose the presence of air-borne spores of obligate parasites or spores of some other fungi as mushrooms and wood-rotting fungi which may often be present in considerable numbers, but they may considerably increase the value of the surveys now being conducted.

Original Articles

INTRAMURAL DISSEMINATION OF SPORES OF HORMODENDRUM RESINAE*

CLYDE M. CHRISTENSEN,† ST. PAUL, MINN.

THE AIM of the work here reported was to determine the pattern of dispersal of fungus spores liberated within a building. By liberating, at a single point in the building, spores of a fungus definitely known not to be present in the air in detectable numbers from other sources, and by using a medium upon which these spores germinate and form visible colonies quickly, but upon which other fungi do not grow, it was possible to trace the travel of these spores throughout the building, to determine the approximate spore load in the air in various parts of the building at various times after the spores were liberated, and to determine how long appreciable numbers of spores remained in the air.

MATERIALS AND METHODS

Organism used.—The fungus used in these tests was *Hormodendrum resinae*. As described by Christensen, Kaufert, Schmitz, and Allison,[1] this fungus, in nature, is largely restricted to wood impregnated with coal tar creosote. Creosoted wood blocks, and agar containing creosote have been exposed from a few hours up to several days, indoors and out, at various times of the year, and on numerous occasions during the last ten years, and subsequently incubated for a week or more; no colonies of *H. resinae* have developed on these. This is fairly convincing proof that the fungus is not a normal inhabitant of the air. With the supporting evidence from the controls used in the tests described below, the possibility of contamination from unknown sources, in these experiments, would seem to be eliminated.

When grown on malt agar, *H. resinae* produces an abundance of powdery spores that can be readily brushed into the air, especially if the agar culture is allowed to dry for several hours before the spores are brushed off. The spores are a few microns in length, and in still air in an enclosed tube fall only a few centimeters per minute, and so are suited to transport by even slow-moving air currents. They retain their viability for days or weeks at the relative humidities ordinarily encountered within occupied buildings. So far as is known, the fungus is innocuous to man, animals, or plants, and therefore the liberation of considerable numbers of its spores into the air should result in no harm or inconvenience. In combination with the selective medium described below, the characteristics of this fungus make it very suitable for use as a "tracer" or "marker" organism in studies such as the present one.

*Paper No. 2556, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, Minn.

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Selective medium.—To prepare the medium 20 Gm. of malt extract and 20 Gm. of agar were placed in 1 liter of distilled water, and the mixture boiled until the agar was dissolved. Five hundred ml. of this were poured into each of two 16 oz. medicine bottles, and autoclaved for fifteen minutes at 15 lbs. steam pressure. The liquid agar was cooled to approximately 60° C. in a water bath, and coal tar creosote equal to 0.5 to 0.75 per cent of the volume of the medium added. (Since coal tar creosotes differ greatly in composition, and are not standardized, one might, using different creosotes, need to use either slightly more or slightly less than the amounts here given.) The bottles were then shaken violently to emulsify the creosote in the agar, and the medium quickly poured into cool, sterile Petri dishes. After pouring a few dishes, the bottle of medium was shaken to maintain a fairly uniform emulsion of creosote.

This medium, hereafter called creosote agar, has the virtue of excluding almost all organisms but *H. resinae*. Other than an occasional colony of Penicillium, which usually can be easily distinguished from *H. resinae* without the use of a microscope, the only organisms found to develop on it have been a bacterium often isolated from creosoted wood along with *H. resinae*, and an unidentified mold isolated from asphalt street paving. Neither of the latter two have been isolated from the air. The writer has strewed soil, house dust, and decomposing plant debris on this agar, and not obtained a single colony of any organism. Since such materials abound in microorganisms of many kinds, this is evidence that the medium is sufficiently selective to serve the purpose for which it has been used in these tests. Colonies of *H. resinae* ordinarily develop rapidly enough on it, at temperatures of 20 to 25° C., to become macroscopically visible within three to four days.

Building in which tests were made.—Most of the tests were made in the Plant Pathology building on the St. Paul campus of the University of Minnesota. The building consists of four stories, with a hallway through the center of each floor, and rooms on either side of the hallways. At both ends of the hallways, interior stairways extend from the first to the fourth floors. The heat is from steam radiators placed against the walls near the floors, and there is no forced air circulating system.

Experimental procedure.—Cultures of *H. resinae* were grown on malt agar in Petri dishes or test tubes. When 2 to 4 weeks old, the surface of such cultures were covered with powdery spores. Before the spores were liberated, dishes of creosote agar were exposed for ½ hour at various stations throughout the building, then covered and stored in the laboratory. These served as controls. In none of the tests did any colonies develop on the dishes exposed before spores were liberated. Another set of dishes containing creosote agar were then exposed, usually on all four floors, in the hallways, and in various offices and laboratories. As soon as these dishes were in place, a portion of the agar culture of *H. resinae* with a surface area of 1 to 4 sq. cm. was removed from a test tube or Petri dish, allowed to dry for a short time, and spores were brushed from the

surface by means of a bit of dry sterile cotton wound around a wire inoculating needle. In most tests, the spores were liberated within a room on the first floor, with the door to the hallway open.

At regular intervals after the spores had been brushed into the air, successive sets of plates were exposed at all stations, those previously exposed being covered and stacked in the laboratory. After liberating the spores and before handling any of the subsequent lots of plates, the operator changed his clothing, washed his hands thoroughly, and put on autoclaved gloves. This was to eliminate the possibility of contaminating the dishes from spores borne on the person. In some tests, one operator liberated the spores, while others, at the different stations, exposed the culture dishes at various times.

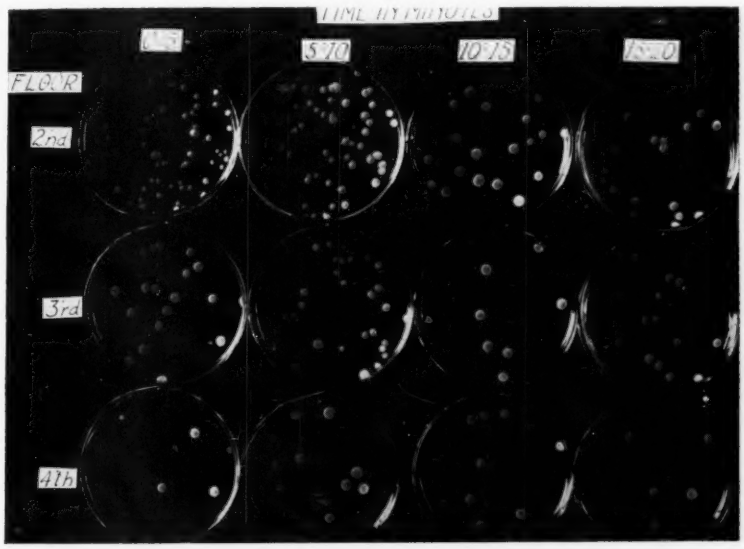

Fig. 1.—Colonies of *Hormodendrum resinae* that developed on plates of creosote agar exposed for successive five-minute periods on the second, third, and fourth floors of the Plant Pathology building after spores had been released in a room on the first floor.

RESULTS

Results of two typical exposures, out of several of those made over a period of several years, are given in Tables I and II, and the colonies which developed on plates exposed in one test are illustrated in Fig. 1.

TABLE I. NUMBER OF COLONIES OF HORMODENDRUM RESINAE ON PLATES OF CREOSOTE AGAR EXPOSED IN SUCCESSIVE FIVE-MINUTE PERIODS ON THE SECOND, THIRD, AND FOURTH FLOORS, AFTER SPORES WERE LIBERATED IN THE FIRST-FLOOR HALLWAY

FLOOR	NUMBER OF COLONIES PER DISH			
	FIRST 5 MINUTES	SECOND 5 MINUTES	THIRD 5 MINUTES	FOURTH 5 MINUTES
2	85	70	27	20
3	20	33	9	16
4	4	17	10	7

TABLE II. NUMBER OF COLONIES OF HORMODENDRUM RESINAE ON PLATES OF CREOSOTE AGAR EXPOSED DIFFERENT PERIODS OF TIME AFTER LIBERATING SPORES IN ROOM ON FIRST FLOOR

LOCATION OF EXPOSED PLATES	NUMBER OF COLONIES PER DISH							
	0-5 MIN.*	5-10 MIN.	10-20 MIN.	20-30 MIN.	30-60 MIN.	60-120 MIN.	120-240 MIN.	TOTAL
First floor hall	228	49	43	31	26	15	5	397
Second floor room, door closed	0	0	1	1	3	10	2	17
Third floor greenhouse connected to main bldg. by 30 ft. passage	0	0	0	0	1	0	1	2
Third floor laboratory, door open	8	0	5	19	55	28	8	123
Third floor hall	0	8	70	35	44	12	0	169
Fourth floor hall	68	78	50	34	51	7	3	291
Fourth floor room, door open	0	0	2	3	7	14	5	31
Totals	304	135	171	123	187	86	24	1030

*Number of minutes after spore release.

In another test, spores were liberated in a laboratory, approximately 20 × 40 feet, on the first floor, in front of an electric fan, with the door closed except when the operator was entering or leaving to uncover or cover dishes. The results are given in Table III.

TABLE III. NUMBER OF COLONIES OF HORMODENDRUM RESINAE ON PLATES OF CREOSOTE AGAR EXPOSED AT SUCCESSIVE INTERVALS IN CLOSED ROOM IN WHICH SPORES WERE LIBERATED

TIME IN HOURS FROM SPORE LIBERATION TO BEGINNING OF EXPOSURE	DURATION OF EXPOSURE IN HOURS	NUMBER OF PLATES EXPOSED	AVERAGE NUMBER OF COLONIES PER PLATE
0	1.5	4	600
1.5	1.5	3	25
5	1	2	2
6	12	2	5

Spores were also liberated in the home of the writer, during the winter, when warm air was being circulated intermittently by a fan in the heating system. No windows were open during the course of the test. The results are given in Table IV.

TABLE IV. NUMBER OF COLONIES OF HORMODENDRUM RESINAE ON PLATES OF CREOSOTE AGAR EXPOSED AT VARIOUS INTERVALS AFTER LIBERATING SPORES IN LIVING ROOM OF WRITER'S HOME. ALL PLATES EXPOSED APPROXIMATELY 5 FEET ABOVE FLOOR

TIME IN HOURS FROM SPORE LIBERATION TO BEGINNING OF EXPOSURE	EXPOSURE IN DURATION OF HOURS	LOCATION OF PLATES	NUMBER OF COLONIES PER PLATE
0	2.5	First floor, living room	550
		Second floor, bedroom	160
3	1	Living room	2
3	4	Bedroom	0
24	12	Living room	8
36	12	Living room	5
48	1	Living room	2

DISCUSSION AND CONCLUSIONS

The most interesting fact gathered from these tests is that spores liberated in a room on the first floor of the Plant Pathology building were carried in con-

siderable numbers throughout the second, third, and fourth floors within a relatively few minutes. The experiments were made during the winter, when convection currents due to artificial heating were greater; even so, the speed and thoroughness with which the spores invaded all rooms with open doors was surprising. Very few colonies developed on dishes exposed in rooms with the doors kept closed during the time the dishes were uncovered.

In most of the tests described above, and in numerous others made since then, very few or no colonies have developed on dishes exposed twelve hours or more after spores were liberated. Those spores recovered in the writer's home two days after spores were liberated may well have come from dust dislodged from the air filter in the air circulating system, since dust taken from this filter more than a week after spores had been liberated, and scattered on creosote agar, gave rise to a very large number of colonies of *H. resinae*. The evidence available suggests that in a building such as that in which most of the tests were made, air-borne contaminants do not linger indefinitely, but largely disappear within a few hours at most. In one test, Petri dishes of creosote agar were exposed near an electric fan in a laboratory where spores had been liberated several days before. The fan presumably stirred up dust on walls, floors, tables, and other furniture. No colonies developed on the culture dishes. The fact that the spores could survive for some time in dust caught in the air filter in the writer's home, and the added fact that some of the spores remained viable for several years on dry material in the laboratory, suggest that the absence of colonies on these dishes was not due merely to death of spores by desiccation or other means. More probably, the circulation of air through the Plant Pathology building was sufficient to carry most of the spores out of doors in a short time. Those that remained probably adhered so firmly to the surfaces on which they landed that they were not easily dislodged.

If the spores of *H. resinae* are carried in large numbers throughout a four-story building within a few minutes after they are liberated in a room on the first floor, and enter in large numbers all of the rooms to which the air itself has free access, other air-borne fungi are probably spread just as effectively. Bakeries and other food processing plants where mold contamination is a problem might well institute studies to determine the major sources of air-borne contamination. Wherever air-borne dissemination of fungi within buildings is known or suspected to be of significance, whether in homes, hospitals, or factories, the above results might be considered.

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PASSIVE TRANSFER OF COLD URTICARIA*

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THE occurrence of urticaria due to cold was described by Duke in 1925[1] as one of the manifestations of physical allergy and has since been reported by many authors. The studies of Lewis[2] suggested that the development of urticaria caused by such physical agents is mediated by H-substance similar in physiologic activity to histamine. Further evidence that a histamine-like substance is involved in the reaction was offered by Horton, Brown, and Roth[3] who showed that development of cold urticaria released into the circulation a substance that produced tachycardia, lowered blood pressure, and increased secretion of free acid in the stomach. More recently antihistamine drugs have been reported to relieve the symptoms of cold allergy.[4, 5]

Evidence of an antibody mechanism in cold urticaria is less conclusive. Antibodies reacting with isoantigens at temperatures near 0° C. are familiar in cold agglutinins and cold hemolysins. The presence of cold agglutinins in high concentration is occasionally manifested clinically by vasomotor symptoms, but not cold urticaria. Harris, Lewis, and Vaughan[6] described cold urticaria associated with cold hemolysins in cases of syphilis. The serum of such a patient locally sensitized the normal skin of another syphilitic patient so that it reacted to cold with the development of urticaria. The skin-sensitizing substance, which they called dermolysin, was removed from the serum by adsorption with erythrocytes in the cold, suggesting it was identical with the cold hemolysin.

Cold urticaria has been reported in patients with cryoglobulinemia associated with various systemic diseases, although purpura and other types of skin sensitivity to cold are more frequent.[7]

The majority of patients with cold urticaria are otherwise healthy and do not have syphilis or diseases producing cryoglobulinemia. In a number of instances the sera of such patients have been reported to sensitize passively normal skin to cold, but a large number of negative reports have cast doubt on the occurrence of passive transfer of this type of reaction.[8, 9] Walzer, in a review of the subject, concluded that the evidence of passive transfer of simple cold urticaria was questionable.[8]

The present report is of studies of a case of cold urticaria in an otherwise healthy patient whose serum readily transferred sensitivity to normal skin, with observations on the properties of the active factor in the serum and an unsuccessful attempt to isolate an antigen from chilled normal skin.

CASE REPORT

J. S., a 42-year-old man, was seen June 6, 1949, because of recurent hives for 6 weeks on exposure to cold. There was a family history of allergy, the patient's brother and 2 of the patient's children having allergic rhinitis. His general health had been good in the past,

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although he had had hay fever in the grass and ragweed pollen seasons and nonseasonal rhinitis on contact with horses for many years. There was no history of syphilis. On several occasions he had noted a few scattered hives after a hot bath but he had no unusual reaction to cold before or during the preceding winter, part of which he spent in New York City.

In April, 1949, while in Florida, he noted an itching hive on his leg which progressed to an isolated purpuric spot 2 to 3 cm. in diameter and gradually faded over a period of 2 weeks. This was thought to be the bite or sting of an unidentified insect or jellyfish. During the 6 weeks after this occurrence, he noted on 3 occasions that his face became swollen and itchy after exposure to cold wind, and that his lip swelled when he held ice against it.

On June 4, 1949, he swam for a few minutes in cold ocean water near New York. Almost immediately after leaving the water, his entire skin surface became itchy and red, and in a few minutes he became weak and collapsed. After rest, Pyribenzamine, and whiskey, he gradually recovered but felt weak the remainder of the day. No hemoglobinuria was noted.

Physical examination on June 6 showed him to be well developed and well nourished, with a blood pressure of 155/110, but showed no other abnormal findings. On subsequent examinations the blood pressure was within normal limits. Dermographism was not demonstrable.

The blood Kline, Mazzini, and Wassermann reactions were negative; the blood group was O. Plasma proteins were 7.6 Gm. per 100 ml.; gamma globulin by the method of Wolfson, Cohn, Calvary, and Ichiba[10] was 0.98 Gm. per 100 ml. The test for cold agglutinins was negative in dilutions of 1:10 to 1:320. The urine was normal. Skin tests with the principal allergens showed marked reactions to timothy pollen, ragweed pollen, horse dander, and house dust. Other inhalants and common foods showed no significant reactions. Intracutaneous tests with histamine acid phosphate gave normal reactions.

The existence of a high degree of cold allergy was confirmed by the observation that application of an ice cube to the skin of the forearm for a period of 10 seconds resulted in the formation of a distinct wheal.

The patient was advised to take a brief cold shower daily and Chlor-Trimeton Maleate, 4 mg. twice a day, and warned of the risk of swimming in cold water. Injections of ragweed pollen extract for hay fever were started.

He spent the summer in Rhode Island where he cautiously tried ocean bathing and found that wading in water below 60° F. (approximately 15° C.) caused itching of the skin of the legs, but that brief immersion in warmer water caused no reaction. He was next seen September 9, at which time repetition of the tests with ice showed a slight decrease in sensitivity, contact for 15 seconds producing a definite urticarial reaction.

On that date, the serum protein was 8.8 Gm. per 100 ml., albumin 6.63 and total globulin 2.1. Electrophoresis of the serum in the Tiselius apparatus showed no unusual components; the normal protein fractions were present in essentially normal amounts. As a test for cryoglobulin, serum was separated at 37° C., then chilled 7 days at 4° C. This did not cause a precipitate to form. A test for cold hemolysins by the method of Mac Kenzie gave no reaction.[11]

During October he made a trip to Europe without noticeable symptoms and was again seen in November. At that time the skin reacted after contact with ice for 20 seconds. During December while in New York, he noted itching of the face, without swelling, after exposure to cold. On Jan. 4, 1950, whealing of the skin occurred only after 40 seconds contact with ice.

Effect of Cold on the Patient's Skin.—As previously noted, on June 6, application of an ice cube to the skin of the forearm for 10 seconds produced a definite urticarial wheal which developed within 8 minutes of the removal of the ice. Contact with ice for 3 to 5 seconds produced erythema with slight whealing. Exposure for 2 seconds had no visible effect. Periods of contact from 15 seconds to 1 minute produced increasingly marked whealing. Contact longer

than one minute caused no greater response. In all cases, the wheal was strictly limited to the area of contact, without pseudopod formation, but with a surrounding zone of erythema 2 to 3 cm. wide. When a site which had reacted was retested the following day, the reactivity was decreased, but exposure to ice for four minutes produced a maximal response.

A slight decrease in the cold sensitivity was demonstrable during the seven months of observation. On June 6, contact with ice for 10 seconds produced a definite hive. On September 9, contact for 15 seconds was effective, and on November 16, 20 seconds' exposure was required. On Jan. 4, 1950, contact with ice for 30 seconds produced only erythema of the skin, 40 seconds slight whealing and 60 seconds a definite wheal.

Effect of Antihistamine Drugs.—In seeking an effective palliative medication the effects of 3 antihistamine drugs were tested on different occasions: Chlor-Trimeton Maleate, 4 mg., and Neo-Antergan, 100 mg., orally, and Benadryl, 20 mg., intramuscularly. In each case the threshold of the whealing reaction to contact with ice was determined, the drug administered, and the tests with ice repeated 30 minutes after the oral medication or 15 minutes after intramuscular injection. In each case the reactions after medication were identical with the control observations made the same day, none of the 3 drugs producing a measureable change in reactivity.

Passive Transfer.—Into a site in the skin of the forearm of a normal person, 0.25 ml. of fresh serum obtained June 9 from the patient, J. S., was injected intracutaneously. Into a control site was injected the same amount of serum from another patient allergic to ragweed pollen but not to cold. Twenty-four hours later ice was applied to both sites for 6 minutes. Within 5 minutes of the removal of the ice, a definite wheal 2.5 × 3 cm., with surrounding erythema, but without pseudopod formation or itching, appeared at the site (Fig. 1) of injection of the serum of J. S. The control site showed no reaction. Sites made with both sera reacted to intracutaneous injection of 0.025 ml. of ragweed pollen extract, 1,000 protein nitrogen units per ml.

Similar urticarial reactions to cold were demonstrated with samples of serum drawn from the patient, J. S., on June 9, September 10, and September 22 in tests on 7 different normal individuals. The results obtained with these 3 samples of serum were essentially identical and these sera were used in tests described subsequently. Sera obtained on November 4 and November 16 were less active but produced definite wheals if sites were tested within 3 hours. Serum taken Jan. 4, 1950, caused definite whealing in 1 of 2 normal subjects tested 2 hours after injection of the serum.

Injections of 0.1 to 0.3 ml. of each of the first 3 samples of serum consistently produced sensitization, the size of the wheal varying with the amount used; even 0.1 ml. of a 1:10 dilution of fresh serum produced a distinct wheal. The reaction could be elicited 2 to 3 hours after the injection of serum, as soon as the irritation produced by the intracutaneous injection had subsided or (with serum less than 4 days old) 24 hours later. Application of ice for periods varying from 6 to 14 minutes produced similar reactions. In most tests contact

was for 10 minutes. Wheals appeared only after the skin again became warm. This could be hastened by placing the arm in a water bath at 35° C. or by applying the palm of the hand to the site, but neither procedure was essential.

In order to test the effect of stimulating the patient's skin on the sensitizing power of the serum, 2 samples of serum were taken on the same day before and after 9 large urticarial wheals were produced on the forearms by applying ice. The 2 samples were equally active in passively sensitizing normal skin.

When the same test subject was used for several tests, care was taken to use a different skin site on each occasion. However, the 2 subjects used for the largest number of tests over a period of weeks both became resistant to sensitization even with fresh serum that was highly active in the skin of other individuals. The nature of this change was not apparent. Serum from the most "resistant" test subject did not form a precipitate with the sensitive serum (J. S.) in ring tests, nor did the "resistant" serum lessen the sensitizing activity of serum J. S. when mixed with it in equal volumes and placed in the refrigerator overnight.

Fig. 1.—Passive transfer of cold urticaria. Upper site sensitized with 0.25 ml. of serum J. S., lower site with 0.25 ml. of serum from another ragweed-sensitive patient. After 24 hours, ice applied to both sites for 6 minutes. Wheal 2 × 3.5 cm. at sensitized site; no reaction at control site.

Inactivation of Serum by Age and Heat.—With freshly separated serum, reactions could be demonstrated by testing sites 24 hours after injection of the serum. Cooling the serum to 4° C. in a refrigerator did not affect its activity. After 4 days' storage at room temperature or in the refrigerator, the serum no longer gave distinct urticarial reactions if 24 hours elapsed between injection and testing with ice, but definite reactions could be elicited if the sites were tested 2 to 9 hours after injection. Serum stored in the refrigerator for 1 month or more showed only feeble activity.

Heating fresh serum one-half hour in a water bath at 56° C. affected its activity only slightly, but 4 hours at the same temperature destroyed its potency. Addition of 0.05 ml. of a 1:5 dilution of fresh pooled guinea pig serum to 0.15 ml. of heated serum did not restore its activity (Table I). Heating four hours at 56° C. also destroyed the activity of the serum in passively sensitizing normal skin to ragweed pollen extract.

TABLE I. EFFECT OF HEATING AT 56° C. ON COLD-SENSITIZING FACTOR

PREPARATION OF SERUM	REACTION IN SITE PREPARED WITH SERUM	REACTION IN SITE PREPARED WITH SERUM AND GUINEA PIG COMPLEMENT
Serum unheated	Wheal 1.6 × 2.5 cm.	Wheal 1.8 × 2.2 cm.
Serum heated ½ hour	Wheal 1.2 × 1.2 cm.	Wheal 1.6 × 1.6 cm.
Serum heated 4 hours	0	0

Exhaustion of Sites by Testing.—Passively sensitized skin sites, which had once reacted to application of cold, did not react to a second stimulation with ice after the first response had subsided. Exhaustion of the reaction to cold did not affect the reaction of sites to intracutaneous tests with ragweed pollen extract, 1,000 protein nitrogen units per ml., suggesting that the factor producing sensitization to cold was independent of the skin-sensitizing antibody for ragweed pollen.

In order to neutralize the skin-sensitizing antibody for ragweed pollen, a portion of serum was mixed with an equal volume of ragweed pollen extract, 5,000 protein nitrogen units per ml., and a corresponding control mixture of serum and saline prepared. Skin sites sensitized with the 2 mixtures both reacted to cold; a site prepared with the serum-ragweed mixture did not react to test with ragweed pollen extract, while a site sensitized with the control mixture did.

Attempts at Reversed Passive Transfer.—Reversal of the usual procedure of passive transfer by injecting the sensitive serum into previously chilled normal skin might be expected to produce a specific reaction, if cold liberated from normal skin a substance (antigen) reacting with the serum. Attempts at reversed passive transfer with the serum J. S. were made on 4 normal test subjects. In each case a portion of the skin of the forearm was chilled by applying ice for 10 minutes. As soon as the ice was removed, 0.05 to 0.15 ml. of the sensitive serum was injected into the chilled skin and an equal volume into uncooled skin. The results were similar in all 4 trials. Each intracutaneous injection of serum produced a slight wheal and redness, but there was no significant difference in the reactions produced in the chilled and normal areas.

Since the activity of sensitized sites had been shown to be exhausted by one reaction to cold, the sites made in 2 test subjects in the attempt at reversed passive transfer were retested 5½ hours later after the irritation caused by the serum injections had subsided. The test and control sites reacted equally, indicating that the activity of the serum had not been exhausted by injection into the chilled skin (Table II).

TABLE II. ATTEMPT AT REVERSED PASSIVE TRANSFER

SENSITIVE SERUM 0.15 ML. INJECTED INTO	IMMEDIATE REACTION	REACTION OF SITE TO ICE 5½ HOURS LATER
Skin chilled with ice for 10 minutes	±	Wheal 1.6 to 1.6 cm.
Skin not chilled	±	Wheal 1.6 × 1.6 cm.

Electrophoretic Fractionation of Serum.—Ten milliliters of undiluted serum adjusted to pH 7.4 with phosphate buffer was subjected to electrophoresis in a Tiselius cell. After the components were separated, 4 samples (0.5 to 0.8 ml. each) were withdrawn corresponding to (1) pure albumin, (2) albumin and alpha globulin, (3) beta and gamma globulin, and (4) pure gamma globulin. Merthiolate was added to each sample to a strength of 1:10,000. Sites were prepared in the skin of 3 test subjects by intracutaneous injection of 0.1 or 0.2 ml. of each fraction. On testing with ice, none of these sites reacted. Sites similarly prepared with the residual material from the electrophoretic cell showed definite reactions, showing that the activity had not been destroyed by the procedure.

The same serum was again run in the Tiselius cell and somewhat larger amounts (1 to 2 ml.) of the same 4 fractions removed. The protein content of the albumin fraction was found to be 0.34 Gm. per 100 ml., that of the albumin and alpha globulin fraction 2.3, and that of the gamma globulin fraction 0.23. The entire third fraction (beta and gamma globulin) was used in passive transfer tests and none remained for analysis. As with the previously obtained fractions, sites prepared in normal skin by intracutaneous injection of each fraction did not respond to chilling with ice. After testing with ice, the sites were retested with 0.025 ml. of low ragweed pollen extract (1,000 units/ml.). As reported by Newell, Sterling, Oxnam, Burden, and Krejci[12] only the sites prepared with gamma globulin reacted to pollen extract.

Addition of 0.05 ml. of fresh pooled guinea pig serum 1:5 to 0.15 ml. of each fraction failed to restore the property of sensitizing to cold. In the case of the albumin and gamma globulin fractions, the addition of 0.05 ml. of fresh normal human serum to 0.15 ml. of electrophoretic fraction was also tried, and likewise failed to restore activity.

A mixture of 0.05 ml. of each of the 4 fractions sensitized normal skin so that a 1.2 cm. wheal was produced by stimulation with ice 3 hours later. A mixture of 0.1 ml. of the second fraction (albumin and alpha globulin) with an equal volume of the third fraction (beta and gamma globulin) gave a stronger reaction with a 1.5 cm. wheal. Mixtures of other combinations (pure albumin with beta and gamma globulin; albumin with pure gamma globulin; albumin and alpha globulin with pure gamma globulin) gave negative results (Table III). The amount of material available did not permit testing all possible combinations.

Because of the marked differences in protein content of the 3 fractions analyzed and the lack of an analysis of the other fraction, definite conclusions as to which of the protein components of the serum were involved in the

reaction are impossible. However, it appeared that transfer of cold sensitivity depended upon the presence of more than 1 component, in contrast to the transfer of ragweed pollen sensitivity which was accomplished with pure gamma globulin.

TABLE III. PASSIVE TRANSFER OF COLD SENSITIVITY BY ELECTROPHORETIC FRACTIONS OF SERUM

FRACTION	PROTEIN COMPONENTS	PROTEIN CONTENT GM./100 ML.	REACTION OF SENSITIZED SITE TO CHILLING
1	Albumin	0.34	0
2	Albumin + alpha	2.3	0
3	Beta + gamma	---	0
4	Gamma globulin	0.23	0
1 + 3	Albumin, beta and gamma globulin	---	0
1 + 4	Albumin, gamma	0.28	0
2 + 3	Albumin, alpha, beta, gamma	---	Wheal 1.5 × 1.5 cm.
2 + 4	Albumin, alpha, gamma	1.7	0
1-2-3 + 4	Albumin, alpha, beta, gamma	---	Wheal 1.2 × 1.2 cm.
Residue in cell	---	---	Wheal 1.6 × 1.9 cm.
Original serum			Wheal 2.5 × 3 cm.

Adsorption of Active Material From Serum With Skin Cells.—A piece of fresh normal human skin excised in the course of a surgical operation was frozen with carbon dioxide and cut into sections 20 micra thick with a microtome. To approximately 0.5 c.c. of such sections was added 1.0 ml. of sterile saline solution and the mixture allowed to stand overnight in the refrigerator. After centrifugalization the supernatant fluid was removed and 0.5 ml. of serum J. S. added to the packed skin cells. Another portion of the serum was added to an equal volume of washed packed Type O red cells. Both tubes were shaken and again placed in the refrigerator at 4° C. for 24 hours. At that time, the sera were removed by centrifugalization at 0° C., and 0.15 ml. of each sample injected intracutaneously in each of 2 normal persons. Control sites were prepared simultaneously with 0.15 ml. of untreated serum J. S. After 3 hours, the sites were tested by applying ice for 10 minutes. The sites prepared with serum "adsorbed" with skin cells showed slight redness but no wheals, those prepared with serum "adsorbed" with red cells and with untreated serum showed definite urticarial reactions, indicating that the sensitizing factor had been fixed by excised skin cells in the cold, but not by Type O red cells (Table IV). When tested 24 hours later with ragweed pollen

TABLE IV. ADSORPTION OF SENSITIZING FACTOR BY EXCISED SKIN

SERUM USED TO PREPARE SKIN SITE (0.2 ML.)	REACTION OF SITE TO CHILLING 3 HOURS LATER
Untreated serum	Wheal 2.2 × 2.0 cm.
Serum adsorbed with skin cells	No wheal
Serum adsorbed with Type O red cells	Wheal 2.2 × 2.8 cm.

antigen (0.025 ml. of extract containing 1,000 protein nitrogen units per ml.) all sites reacted essentially equally, indicating that the skin-sensitizing antibody for ragweed was not adsorbed in vitro by skin cells.

Attempt to Isolate Antigen From Frozen Skin.—If an antigen were liberated from normal skin by cold and reacted with the patient's serum, it might be expected to be present in the saline which had been used to wash the frozen skin cells in the adsorption experiment described in the preceding section. As a test for such an antigen, skin sites prepared by the injection of 0.1 ml. of serum J. S. and control sites injected with an equal amount of normal serum were tested after 4 hours by intracutaneous injection of 0.025 ml. of the supernatant fluid obtained after washing the skin cells with saline. This material produced a slight (0.4 cm.) wheal in both sites. The reaction in the site prepared with serum J. S. was no greater than that in the control site. There was no evidence that freezing normal skin liberated an antigen which would react with the serum J. S. at body temperature.

DISCUSSION

The patient had typical and severe cold urticaria with the generalized reaction to bathing in cold water described by Brown and Horton.[13] The degree of sensitivity to cold was unusual, contact with ice for 10 seconds producing an urticarial wheal. In most cases with similar symptoms contact with ice for 4 to 10 minutes is necessary to produce a similar reaction. During the period of 7 months of observation, the patient's sensitivity appeared to decrease definitely but remained marked. In June, 1949, contact with ice for 10 seconds produced a definite wheal, while in January, 1950, contact for 40 seconds was necessary. Whether this change was spontaneous or resulted from daily cold baths during most of the period was not determined. Antihistaminic drugs (Chlor-Trimeton Maleate, 4 mg., Neo-Antergan, 100 mg., by mouth, and Benadryl, 20 mg., intramuscularly) did not affect the reaction. Their inefficacy, in contrast to previous reports,[4, 5] may have been due to the extreme sensitivity of this patient.

There was no evidence of syphilis, cold hemolysins, cold agglutinins, or cryoglobulinemia. The onset of symptoms within a few weeks after a lesion which was presumed to be the bite or sting of an unidentified insect or jellyfish is of interest. Such a temporal relationship to a sting or bite has been reported in cases of urticarial reaction to sunlight,[17, 18] but not in cold urticaria. The *possibility* of a causal relationship between the bite and physical allergy remains *uncertain*.

The present case was unusual not only in the degree of sensitivity to contact with ice, but also in the marked activity of the patient's serum in sensitizing normal skin to cold. Such passive sensitization is only occasionally demonstrable, usually of brief duration (2 to 5 hours) and the reactions small.[8] With the sera obtained in June and September, 1949, wheals 2 to 3 cm. in diameter were demonstrable on testing sites 24 hours after sensitization. Samples of serum obtained in November, 1949, and January, 1950, were definitely less active. All samples of serum decreased in potency when stored at room temperature or at 4° C. for 4 days or more.

The urticarial reactions elicited in the patient's skin and in passively sensitized normal skin by cold resembled, in general, those produced in sensitive individuals by material antigens. The resemblance suggests that the reaction might be mediated by an antibody present in the serum. The possibility of such sensitizing antibody was perhaps greater since the patient was known to be allergic to ragweed and grass pollens. The thermolability of the cold-sensitizing factor in the serum corresponded to that of the skin-sensitizing antibody for ragweed, both being destroyed by heating the serum 4 hours at 56° C. However, the reactions to cold and to ragweed pollen were shown to be unrelated in exhaustion and neutralization tests.

Since the reaction could be demonstrated on normal skin, it might be supposed that the antigen was liberated from normal skin by cold, a mechanism similar to that suggested in some cases of urticaria produced by ultraviolet light.[14, 15] However, attempts to demonstrate an antigen in an extract of excised frozen normal skin which would react with the serum at body temperature were unsuccessful, as were attempts at reversed passive transfer.

Unlike ultraviolet light,[16] cold is not known to change the specificity of antigens, and in the case of cold hemolysis and cold agglutination, low temperatures are presumed to facilitate the reaction between antibody and a preexisting antigen in the red cells, rather than produce a new antigen. Previous chilling of the red cells does not cause them to react with the cold hemolysin or cold agglutinin when added to it at body temperature. The reactions of the present serum were consistent with those of an antibody reacting at temperatures near 0° C. with an antigen present in normal human skin. The analogy to a cold hemolysin was furthered by the observation that the skin-sensitizing substance was adsorbed from the serum by skin cells in the cold, as cold hemolysins are adsorbed by red cells under similar conditions. However, the serum of this patient differed from that described by Harris, Lewis and Vaughan,[6] which produced both cold hemolysis and cold urticaria, in that no cold hemolysin was present and the factor transferring cold urticaria was not adsorbed by red cells in the cold.

The concept of an antigen fixed in the skin cells might also explain the observation that neither the wheals produced on the patient's skin or those on passively sensitized skin showed the pseudopod formation which almost invariably occurs in wheals of comparable size produced by ordinary dissolved antigens.

The interpretation of the passive transfer tests with electrophoretic fractions of the serums is not clear, but the results strongly suggested that more than one protein component of the serum was involved in the passive sensitization of normal skin. Other antibodies which have been studied by electrophoresis have been found to move as a single component. Since complement was not apparently involved in the reaction, the presence of 2 (or more) components necessary for the reaction might be taken to suggest that both antigen and antibody were contained in the serum. No conclusive evidence on this point was obtained, but there was no precipitation or other evidence of antigen-antibody reaction when the serum was chilled in the absence of skin cells.

SUMMARY

1. A case of marked cold allergy manifested by urticarial reactions of the skin to contact with ice for 10 seconds is described. The reaction was not affected by ordinary doses of antihistamine drugs.

2. The patient was allergic to pollens and horse danders, but apparently free of other diseases, and the serum did not contain cold agglutins, cold hemolysins, or cryoglobulins. The electrophoretic pattern of the serum was normal.

3. Injection of the serum intracutaneously into normal skin produced passive local sensitization demonstrable by an urticarial reaction to contact with ice. The passive sensitization was exhausted by one reaction to cold. Stimulation of the skin of the patient with ice did not affect the sensitizing power of the serum.

4. The skin-sensitizing activity of the serum was only slightly affected by heating one-half hour at 56° C. but was destroyed by heating 4 hours at this temperature. Addition of guinea pig complement to the heated serum did not restore activity.

5. The sensitizing material in the serum was adsorbed by excised skin cells at 4° C., but not by Type O red cells.

6. Attempts to demonstrate reversed passive transfer and to isolate an antigen liberated from normal skin by cold which would react with the serum were unsuccessful.

7. Four fractions were separated from the serum by electrophoresis, containing respectively (1) albumin, (2) albumin and alpha globulin, (3) beta and gamma globulin, and (4) gamma globulin. None of these fractions passively sensitized normal skin to cold and addition of complement did not render them active. Mixtures of all 4 fractions or of the second and third fractions sensitized normal skin, suggesting that the presence of more than one protein component was necessary to produce sensitization.

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HUMAN SKIN SENSITIVITY TO PENICILLINS G, BT, AND O. DEMONSTRATION OF CROSS-SENSITIZATION*

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SENSITIVITY to penicillin as manifested by urticaria, various skin rashes, fever, and other sequelae is observed quite frequently.[1] While in the majority of cases it presents only an inconvenience to the patient, severe reactions have been recorded.[2, 3] Originally, the sensitizing property of penicillin was attributed to impurities in the amorphous (yellow) form of the drug, but crystalline material also has the capacity to sensitize.[4, 5]

"Penicillin" is a generic term and designates a group of compounds that contain an alanine dimethyl cysteine nucleus but which differ in the side chains. Commercial preparations consist predominantly of penicillin G, characterized by a benzyl group in the side chain. However, a variety of substituted penicillins, so-called biosynthetic penicillins, can be prepared by incorporating appropriate precursors in the media in which *Penicillium notatum* (or chrysogenum) is grown.[6] Two such penicillins,‡ "BT" (*n*-butylthiomethylpenicillin) and "AT" or "O" (allylmercaptomethylpenicillin) have been studied to a limited extent.[7, 8] The chief interest in these penicillins is the possibility of decreasing the incidence of sensitization following their use by reason of the straight chain substituent in their structure replacing the benzene ring of penicillin G.

This study was undertaken to determine whether there were any differences in skin sensitivity to penicillins G, BT, and O.

SUBJECTS STUDIED

The test subjects were patients on the wards of two general hospitals. Careful inquiry was made as to previous penicillin therapy, allergic background, and drug sensitivity. Only a few patients with dermatoses were tested and no patient was tested who had received penicillin therapy within the preceding 5 days. Histories of allergic reactions to penicillin were carefully investigated and an effort made to obtain confirmation from medical personnel.

For purposes of evaluation, the subjects have been classified into three groups: Group A, those who never knowingly received penicillin§; Group B, those who had received penicillin; and Group C, those who were known to have shown allergic reactions to penicillin. Of the 270 patients who received penicillin (Groups B and C), the previous penicillin exposure was topical (ointment or aerosol) in 5, oral in 4, and parenteral in 261. From no patient in this study

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§"Penicillin" refers to crystalline penicillin G, since other types of penicillin have been available for investigational purposes only and may be assumed to have been used in a very limited number of individuals.

was the history obtained that reaction followed topical application of penicillin. Fourteen persons in Group C had had urticarial reactions and the remaining 17 had had generalized rashes with pruritus. Clinical reactions were attributable to oral administration in 3, and to parenteral administration in 28 patients.

MATERIALS USED

The sodium salt of penicillin G, the potassium salt of penicillin BT, and the sodium salt of penicillin AT or O (hereinafter referred to as penicillin O) were dissolved in isotonic saline solution to a concentration of 20,000 units per ml. Because the hypertonic solutions that resulted were irritating, distilled water was later substituted for the saline solution. Solutions were used immediately or stored at –20° C. for periods not longer than 2 weeks. The penicillins of the types used in this investigation were as nearly pure as it is practical to manufacture them. Commercial crystalline penicillin G is highly purified but it contains traces of contaminants in the form of other penicillins. Similarly penicillins BT and O contain contaminating traces of other penicillins.

METHODS

Intracutaneous injections of 0.1 ml. containing 2,000 units of the penicillins to be tested were made in widely separated sites on the flexor surface of the forearm. Twenty-four and 48 hours later, erythema and induration at the sites of injection were measured in two diameters. An area of erythema less than 4 mm. in diameter at the end of 24 hours was recorded as a negative skin reaction. Other reactions such as vesiculation, itching, and systemic manifestations were noted.

RESULTS

Of the 276 persons tested with penicillins G and BT, a total of 37, regardless of previous exposure, were reactive to G and 29 were reactive to BT (Table I). It is noteworthy that of the 29 persons reacting to BT penicillin, 28 reacted also to penicillin G.

TABLE I. COMPARISON OF SKIN SENSITIVITY TO PENICILLINS G AND BT

	GROUP A NO KNOWN PENICILLIN THERAPY		GROUP B PREVIOUS TREATMENT WITH PENICILLIN		GROUP C KNOWN REACTION TO PENICILLIN		TOTALS	
RESPONSE TO SKIN TEST	NO. OF PATIENTS	%	NO. OF PATIENTS	%	NO. OF PATIENTS	%	NO. OF PATIENTS	%
+G, +BT	6	4.5	8	7.1	14	53.5	28	10.0
+G, –BT	1	0.7	5	4.4	3	11.6	9*	3.2
–G, +BT	1	0.7	0	0.0	0	0.0	1*	0.3
–G, –BT	129	94.1	100	88.5	9	34.9	238	86.5
Totals	137		113		26		276	

*Deviation in distribution from chance, in these two groups highly significant, p = 0.019.

Within the separate categories (Groups A, B, C) there were no statistical differences in the incidences of positive reactions to penicillins G and BT. In the entire series only 10 persons reacted differentially, that is, 9 were +G, –BT

and 1 was –G, +BT. The likelihood of obtaining this distribution (9:1) between two alternative responses on the basis of chance alone is very remote ($p = 0.019$). It should also be noted that the incidence of positive reactions to both penicillins paralleled the previous exposure to penicillin. Thus the total incidence of positive skin tests in Group A was 5.9 per cent, Group B, 11.5 per cent, and in Group C, 65.1 per cent.

In Table II are presented the results of testing 224 persons with penicillins G and O. Thirty-six patients were positive to penicillin G, 26 were positive to penicillin O, and 12 reacted differentially. Of the latter group, 11 were +G, –O and only one was –G, +O. Here again, the incidence of differential reactions (11:1) departs significantly from expectancy on the basis of chance alone ($p = 0.0059$). In this series, also, there is an obvious correlation between the incidence of positive skin tests and the previous penicillin exposure, in Group A, 4.4 per cent, Group B, 17.3 per cent, and Group C, 65 per cent.

One hundred patients were tested with all 3 penicillins. Although the data on differential responses are too few to be analyzed statistically, certain observations are noteworthy. In this group, a total of 19 patients showed positive reactions; 13 were sensitive to all 3 penicillins, +G, +BT, +O; 4 were +G, +BT, –O; 1 was –G, –BT, +O; and 1 was –G, +BT, –O.

TABLE II. COMPARISON OF SKIN SENSITIVITY TO PENICILLINS G AND O (AT)

RESPONSE TO SKIN TEST	GROUP A NO KNOWN PENICILLIN THERAPY		GROUP B PREVIOUS TREATMENT WITH PENICILLIN		GROUP C KNOWN REACTION TO PENICILLIN		TOTALS	
	NO. OF PATIENTS	%	NO. OF PATIENTS	%	NO. OF PATIENTS	%	NO. OF PATIENTS	%
+G, +O	2	2.2	11	10.0	12	55.0	25	11.2
+G, –O	1	1.1	8	7.3	2	10.0	11*	4.8
–G, +O	1	1.1	0	0.0	0	0.0	1*	0.4
–G, –O	89	95.6	91	82.7	7	35.0	187	83.6
Totals	93		110		21		224	

*Deviation in distribution from chance, in these two groups highly significant, $p = 0.0059$.

DISCUSSION

Crystalline penicillin G has sensitizing properties that may be of serious clinical consequence, and while early reports indicated that the incidence of allergic reactions varied from 0.5 per cent[9] to 2.8 per cent[10] recent data indicate an incidence of 6 to 12 per cent.[5, 11, 12] The increasing incidence of reactions probably reflects the more widespread use of penicillin. In one series[5] a positive skin test (intracutaneous) to crystalline penicillin G was found in 5.7 per cent of the patients who had never received the antibiotic and an incidence of 13.4 per cent in those who had. Similar observations have been noted in this study; the incidence of positive skin tests was increased in persons with previous penicillin exposure, (compare Groups A, B, and C in Tables I and II).

The intracutaneous skin test is recognized as a useful technique for determining sensitivity to a particular antigen, but its reliability in predicting an individual's response to parenteral administration or ingestion of the antigen

can be seriously questioned. Despite the limitations of skin testing, it is the only means other than the clinical history of previous reactions to the antibiotic agent that is available for attempting to predetermine a patient's reaction to therapy with penicillin. A negative skin test does not always indicate freedom from sensitivity but a positive skin test certainly demands cautious use of parenteral therapy; in such cases it is suggested that 2,000 to 5,000 units be injected intramuscularly as a trial dose. Similarly, a clinical history of reaction to penicillin does not preclude subsequent use of penicillin, but caution in instituting therapy is enjoined.

The observation in one series of 33 patients,[4] that 58 per cent of those showing a positive skin test exhibited a generalized reaction to 100,000 units injected intramuscularly, lends weight to the viewpoint that a positive skin test bespeaks caution.

The advantages of having other penicillins devoid of cross-sensitization with penicillin G are obvious. Rose, Harris, Behrens, and Chen[7] sensitized guinea pigs to penicillin G but were unable to sensitize them to penicillins BT or AT (O). They were also unable to demonstrate in guinea pigs any cross-sensitivity between penicillins G and BT or O. These results do not appear consistent with our observations in humans, since many individuals were found reacting positively to all three agents. In addition, the data in Tables I and II demonstrate that not only does the incidence of positive reactions to penicillin G increase with previous exposure, but the incidence of positive reactions to penicillins BT and O increases similarly. Since it is chiefly penicillin G which is being used clinically, and there is little probability that penicillins BT and O occur naturally, it appears likely that cross-sensitivity among these agents exists.

The occurrence of positive skin tests to crystalline penicillin G in persons who have never been exposed to therapy has been repeatedly observed,[1, 5] but there is no agreement as to the explanation of this phenomenon. Many of these persons have been reported to react to trichophyton extracts, and there is the possibility that previous fungus infections may have some relation to penicillin sensitivity. It has been suggested that fungi causing disease in man may produce substances which have a close antigenic relationship to penicillin and that sensitivity to these substances may be aggravated by penicillin.[13, 14] Thus, persons never previously treated with penicillin may show sensitivity the first time they are exposed to it. It is of interest that in children and women in whom the incidence of trichophyton infections is lower than in men, the incidence of penicillin sensitivity is also lower. Those patients in this study not known to have had penicillin previously (Group A) showed an incidence of positive skin reactions of 5.2 per cent, whereas clinically obvious (in some cases florid) epidermatophytosis in the group of patients studied was much more frequent. It would have been desirable to correlate our studies with trichophyton and oidiomycin skin tests, but this was not done.

There were 12 patients in both series who reacted with positive skin tests and denied having previous therapy (Group A, Tables I and II). Scrutiny of the reactions of these 12 patients showed that 6 were +G, +BT, and 2 were

+G, +O; 1 was +G, –BT; 1 was +G, –O; and 2 patients reacted in a manner difficult to interpret, –G, +BT and –G, +O. The possibility of a contaminant of penicillin G in our penicillins BT and O giving these last 2 results is obviously eliminated by the remaining significant data showing differential responses in Groups B and C. Actually, these 2 tests were not markedly positive and we are inclined to regard them as nonspecific reactions, since it is difficult to understand sensitivity to a biosynthetic penicillin in the presence of a negative response to the naturally occurring type. Apart from these 2 cases, the remaining data are readily interpretable. The majority of persons showing skin sensitivity (72.6 per cent) were reactive to both types of penicillin used. A small number (27.4 per cent) were reactive to penicillin G but were not reactive to the biosynthetic penicillins BT or O. It is suggested that in some persons sensitization is due to the nucleus which is common in the structure of these penicillins, while in others the sensitization is attributable to the entire molecule (nucleus and side chain). In the former situation, the patient will react to all penicillins, in the latter case only to a specific type of penicillin.

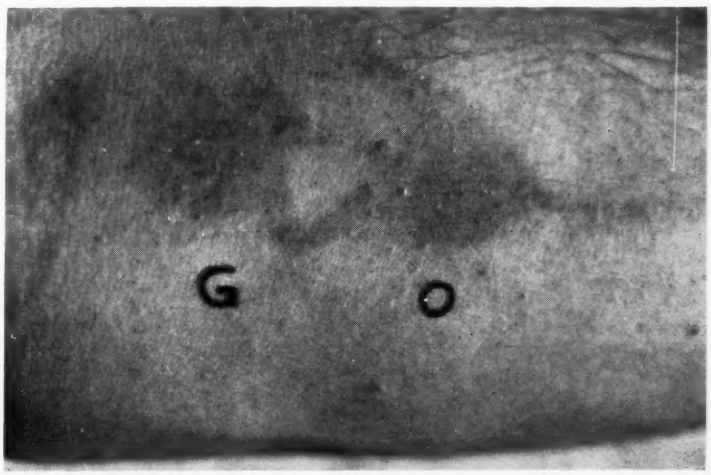

Fig. 1.—Results of intracutaneous skin tests with 2,000 units of penicillins G and O. Patient was a 25-year-old student who had had a generalized reaction to penicillin G 3 months previously. Photograph was taken 14 days after tests were performed. Note pseudopodia radiating from sites of both tests.

It is generally recognized that the severity of skin responsiveness to a test antigen cannot be used to predict the severity with which the patient may react to the antigen when administered by other routes. Many skin reactions to penicillin G were more marked than those obtained with BT or O, but in other instances there was either no difference or, in a few cases, the response to G was less marked. It is noteworthy, however, that persons who showed strongly positive tests to penicillin G (erythema and induration 10 to 20 mm. in diameter) were always positive to penicillins BT and/or O (Fig. 1). On the other hand, in those persons who showed differential responses, skin tests were less markedly positive and in the range of 5 to 10 mm. in diameter.

In no instance was vesiculation or central necrosis seen in a positive reaction. In 2 patients mild generalized reactions with pruritus and rash occurred following skin tests with all 3 penicillins. Both had had previous reactions to intramuscular penicillin therapy; 1, 3 months before and the other, 1 week prior to testing. It is worthy of note that a total of 6,000 units of penicillin elicited a generalized reaction in patients sensitive to the antibiotic.

The necessity of administering penicillin to patients in Group C, those who have already had a generalized reaction, presents the most serious problem that might be solved by having available penicillin that lacked sensitizing properties. The data from Group C (Tables I and II) show that only 2 of 21 patients tested with penicillins BT and G, and only 3 of 26 tested with penicillins G and O manifested a differential response that offered the possibility of substituting penicillins BT or O for the G type. Seventy-three patients, regardless of previous therapy, reacted to penicillin G and of this number, 20 (27.4 per cent) did not react to penicillins BT or O. Thus, the availability of a penicillin that could be used as a substitute for penicillin G in the event of allergic reactions might have definite value in this limited number of instances. It is noteworthy that many patients who exhibit a generalized reaction of rash or even urticaria following penicillin treatment may be continued on therapy, but in some patients continuation of treatment can be disastrous, resulting in exfoliative dermatitis and death. The advent of aureomycin and chloramphenicol which may be substituted for penicillin for therapeutic purposes would seem to lessen the need for such penicillins as BT and O, but in some situations where penicillin is the drug of choice it would be highly desirable to have a biosynthetic penicillin to which patients were not sensitive that could be substituted for penicillin G.

SUMMARY

1. Intracutaneous skin tests were performed on 276 persons using penicillins G and BT and on 224 persons using penicillins G and O (AT).

2. The incidence of positive skin reactions to all 3 penicillins showed an increase in persons with previous exposure to penicillin G, and a greater increase in those with a history of previous general reactions to penicillin G.

3. Positive skin tests to all 3 penicillins were observed in persons who denied ever having received penicillin therapy as well as in those who had received therapy. These data suggest that from the clinical standpoint cross-sensitivity to these penicillins exists.

4. A statistically significant number of differential responses were observed when the same persons were skin tested with penicillins G and BT or with penicillins G and O. The number of instances in which these differential responses occurred was small.

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STUDIES ON TOPICAL THEPHORIN* THERAPY: INDEX OF SENSITIZATION AND EFFECTIVENESS AS AN ANTIPRURITIC

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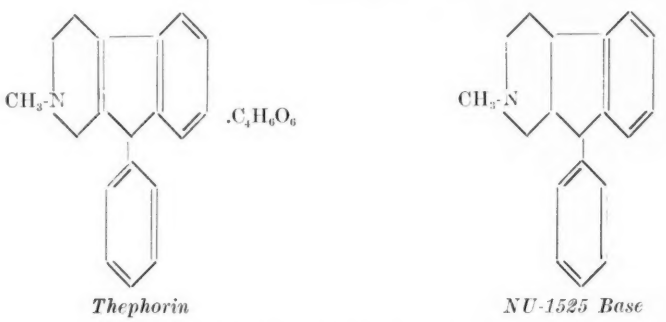
DURING the past few years the use of antihistaminic drugs in the treatment of various dermatoses has increased appreciably. The majority of the histamine antagonists employed are derivatives of ethanolamine, ethylenediamine and phenylpropylamine, such as Benadryl, Pyribenzamine, Histadyl, and Trimeton. Recently, the pyridindene derivative, Thephorin (phenindamine), which is chemically (2-methyl-9-phenyl-2,3,4,9-) tetrahydro-1-pyridindene hydrogen tartrate, has been introduced as an antihistaminic agent. It appears from the structural formulae shown in Fig. 1 that Thephorin is entirely unrelated chemically to the afore-mentioned compounds.

A number of reports have appeared on the topical application of Thephorin.[1-10] Most of the authors stress chiefly the subjective relief from itching and subjective, as well as objective, improvement of chronic pruritic eruptions, mainly localized and disseminated neurodermatitis, and pruritus of the anogenital region.



Thephorin *NU-1525 Base*

Fig. 1.—Structural formulae of Thephorin (phenindamine) and its congener, NU-1525.

The opinions expressed on the sensitizing properties of Thephorin Ointment are at variance. For instance, Shelmire[2] studying 455 patients observed 6 instances of contact-type sensitization, an incidence of 1.31 per cent. Similarly, Laymon et al.,[8] treating 324 cases, 5 of which proved sensitive to the antihistaminic, concluded that "Thephorin Ointment sensitizes only 1.5 per cent of individuals upon which it is used." They distinguished carefully between true eczematous sensitization and nonspecific flares, but cautioned that the percentage of sensitization may change and that time alone will determine its true index. A still lower sensitizing index was reported by Reiss and Kern,[10] who, studying 161 patients, encountered only 1 patient, or 0.6 per cent, who developed sensi-

*Hoffmann-La Roche brand of phenindamine.

†From the Department of Dermatology, Queens General Hospital, Jamaica, N. Y., I. J. Mintzer, M.D., Director, and the Long Island College Hospital and the Department of Dermatology and Syphilology, State University of New York, State University Medical Center at New York, Brooklyn, N. Y.

tivity. Ellis and Bundick,[5] treating 50 patients, obtained positive patch tests with Thephorin Ointment, Thephorin Lotion, or a 2 per cent aqueous solution of Thephorin in 5 subjects. They state that only 3 of these were proved sensitive to the drug itself, but patch tests with an aqueous solution of Thephorin were carried out in only 2 patients. Moreover, the small number of cases studied renders invalid the use of these figures as an index of the allergenicity of the drug. Finally, D'Avanzo[9] reported 74 patients with chronic pruritic dermatoses treated with Thephorin Ointment, 4 of whom developed sensitivity, an incidence of 5.4 per cent. The author, however, states that "Thephorin Ointment has a sensitizing index of 16 per cent," evidently an error in arithmetic. This series, of course, is not large enough to serve as the basis for any conclusions about the true index of sensitization of Thephorin Ointment. Moreover, comment is lacking on the method of determining the presence of sensitization.

Since all workers are agreed that Thephorin Ointment is an effective antipruritic, additional data on its sensitizing property seemed desirable. Therefore, experiments in this direction were undertaken including trials regarding cross-sensitization with other antihistaminic drugs.

TABLE I. RESULTS OF TREATMENT WITH THEPHORIN OINTMENT IN 200 PATIENTS WITH PRURITIC DERMATOSES

DIAGNOSIS	TOTAL NO. PATIENTS	RESULTS				
		GOOD*	FAIR†	POOR‡	FLARES	POSITIVE PATCH TESTS
Atopic dermatitis	35	5	18	12	4	0
Circumscribed neurodermatitis (lichen simplex chronicus)	103	64	10	29	10	10
Anogenital pruritus	21	13	6	2	2	2
Acute urticaria	12	11	1	0	0	--
Acute eczematous contact-type dermatitis	15	0	1	14	8	0
Stasis dermatitis	8	2	2	4	2	0
Lichen planus	4	0	1	3	0	--
Lichen urticatus	2	0	1	1	0	--
Totals	200	95	40	65	26	12

*Good results indicate 75 per cent or more subjective and objective improvement.

†Fair results indicate 25 to 75 per cent improvement.

‡Poor results indicate improvement which could not be considered clinically significant, (i.e., less than 25 per cent) or clinical flares.

The importance of controls in the evaluation of any topical remedy is obvious. Shelmire[2] commented that the relief produced by Thephorin Ointment was much greater and more lasting than that obtained in control tests from the nonmedicated ointment base. Similarly, Reiss and Kern[10] tried placebo ointment, either alternately with Thephorin Ointment or by the method of paired comparison, in 120 patients with pruritic dermatoses who had derived benefit from Thephorin Ointment. Eighty-six of these subjects, or 71.7 per cent, were relieved from their pruritus only when they applied Thephorin Ointment. However, control tests utilizing strictly objective criteria seemed more appropriate and, therefore, were instituted.

The third phase of this study includes trials with topical medication forms of Thephorin other than the ointment in Carbowax 1500, namely, with a 5

TABLE II.

SUMMARY OF FINDINGS IN 12 PATIENTS DEVELOPING TRUE

CASE NO.	AGE	SEX	DIAGNOSIS	DURATION OF THERAPY PRIOR TO APPEARANCE OF FLARE (DAYS)	PATCH TESTS WITH: THEPHORIN OINTMENT	2% AQUEOUS SOLUTION OF THEPHORIN	CARBOWAX 1,500	POSITIVE WITH AQUEOUS THEPHORIN IN DILUTION	NU-1525 OINTMENT
1	58	F	Lichen simplex	15	4+	4+	Neg.	1 to 10,000	+-
2	44	M	Pruritus ani	20	3+	4+	Neg.	1 to 1,000	+-
3	60	F	Lichen simplex	28	4+	4+	Neg.	1 to 1,000	1+
4	62	F	Pruritus vulvae	24	4+	4+	Neg.	1 to 1,000,000	1+
5	31	F	Lichen simplex	38	2+	4+	Neg.	1 to 100	+-
6	39	F	Lichen simplex	29	4+	4+	Neg.	1 to 10,000	1+
7	40	F	Lichen simplex	15	4+	4+	Neg.	1 to 100,000	1+
8	24	F	Lichen simplex	29	3+	4+	Neg.	1 to 1,000	1+
9	28	F	Lichen simplex	17	4+	4+	Neg.	1 to 1,000	1+
10	31	F	Lichen simplex	38	4+	4+	Neg.	1 to 1,000	+-
11	48	F	Lichen simplex	22	4+	4+	Neg.	1 to 10,000	1+
12	49	F	Lichen simplex	45	4+	4+	Neg.	1 to 1,000	1+

Positive reactions were graded from 0 to 4 + as follows: 1 + = erythema; 2 + = erythema and edema; 3 + = erythema, edema, and vesiculation; 4 + = bleb formation.

per cent lotion and a 5 per cent ointment, the base of which consisted of equal parts of Aquaphor and cold cream.

Finally, experiments were carried out with an ointment containing 5 per cent of the hydrobromide of NU-1525 in a carbowax base. As is seen in Fig. 1, the latter compound contains 2 hydrogen atoms more and, thus, one double bond less than the Thephorin base. Trials with NU-1525 seemed of particular interest since this compound shows very slight antihistaminic action, but is as equally effective a local anesthetic as Thephorin to which it is so closely related chemically.[11]

STUDY OF FREQUENCY OF SENSITIZATION

Two hundred patients with various pruritic dermatoses were treated with Thephorin Ointment,* for periods of time ranging in general from 3 weeks to 6 months. Only in cases showing evidence of a flare before 3 weeks was the ointment applied for a shorter period, but in several instances treatment was

*Thephorin Ointment contains 5 per cent Thephorin in a base consisting of Carbowax 1500.

II.

TRUE SENSITIZATION TO THEPHORIN OINTMENT

BENADRYL OINTMENT	PYRIBENZAMINE OINTMENT	TRIMETON OINTMENT	HISTADYL OINTMENT	EFFECT OF INGESTION OF THEPHORIN: 25 MG. T.I.D., P.C., FOR ONE DAY OR UNTIL ERUPTION APPEARED	COMMENTS
Neg.	Neg.	Neg.	Neg.	Generalized maculopapular eruption and flare of previously involved sites 12 hours after first dose	Moderate relief from itching until onset of flare
Neg.	Neg.	Neg.	Neg.	No reaction	Considerable relief from itching until onset of flare
Neg.	Neg.	Neg.	Neg.	Generalized maculopapular eruption and flare of previously involved sites 4 hours after first dose	Some relief from itching until flare occurred
Neg.	Neg.	Neg.	Neg.	Not tested	Considerable relief from itching until onset of flare
Neg.	Neg.	Neg.	Neg.	No reaction	Moderate relief from itching until onset of flare
Neg.	Neg.	Neg.	Neg.	Generalized maculopapular eruption and flare of previously involved sites 2 hours after second dose	Moderate relief from itching until onset of flare
Neg.	Neg.	Neg.	Neg.	No reaction	Moderate relief from itching until onset of flare
Neg.	Neg.	Neg.	Neg.	Generalized maculopapular eruption and flare 2 hours after first dose	Several patches of lichen simplex had almost disappeared with onset of flare. Recurred when Thephorin was discontinued
Neg.	Neg.	Neg.	Neg.	No reaction	Moderate relief from itching until onset of flare
Neg.	Neg.	Neg.	Neg.	No reaction	Same as above
Neg.	Neg.	Neg.	Neg.	Generalized maculopapular eruption and flare 4 hours after third dose	Moderate relief from itching until onset of flare
Neg.	Neg.	Neg.	Neg.	Generalized maculopapular eruption and flare ½ hour after first dose	Same as above

continued for longer than 6 months. Table I groups together cases with identical diagnoses and summarizes the clinical results observed. It can be seen that a total of 135 patients derived benefit from the ointment, an incidence of improvement of 67.5 per cent. It appears also that the lesions of 26 patients (13 per cent) flared and that in 12 of these, or in 6 per cent of the total number of patients treated, patch tests proved the presence of sensitization to Thephorin. Ten of the patients developing contact-type dermatitis to Thephorin had circumscribed neurodermatitis and 2 suffered from pruritus of the anogenital region. Four patients with atopic dermatitis, 8 subjects with an original diagnosis of acute eczematous contact-type dermatitis, and 2 with stasis dermatitis developed flares following treatment with Thephorin Ointment; however, repeated patch tests, including the sites of previous flares, and use tests instituted after complete subsidence of the acute dermatitis were consistently negative in all of them. Three of the 8 patients with acute eczematous contact-type dermatitis developing a flare promptly encountered the same phenomenon following the use of the nonmedicated carbowax base.

As shown in Table II, the flare in the 12 patients who developed sensitization appeared after periods of treatment ranging from 15 to 45 days. It can be seen also that patch tests to Thephorin Ointment as well as to a 2 per cent aqueous solution of Thephorin were positive in all subjects and that they were negative to the carbowax base and to 4 other antihistaminic ointments containing Benadryl, Pyribenzamine, Trimeton, and Histadyl, respectively. Subsequent patch tests with serial dilutions of Thephorin were positive in concentrations of 1:1,000,000 and of 1:100,000 in 1 patient each, in a dilution of 1:10,000 in 3 and of 1:1,000 in 6 subjects, and, finally, in a concentration of 1:100 in 1 patient. The reactions elicited by an ointment containing 5 per cent of a congener of Thephorin, designated NU-1525, were all less marked than those produced by Thephorin itself. The most severe reaction to Thephorin Ointment was observed in a 62-year-old patient with pruritus vulvae (Case 4, Table II). She developed an almost universal eczematous dermatitis. Thephorin Ointment had given her considerable relief, but, after using it 24 days, a small patch of dermatitis appeared on the vulva. Notwithstanding, the patient continued to use the ointment because of its marked antipruritic effect, even when the eruption involved thighs, abdomen, and buttocks. Suddenly the dermatitis became generalized, involving almost the entire cutaneous surface. Under treatment with colloid baths and bland lotions, the eruption subsided in 2 months. She later developed an eczematous contact-type dermatitis about the eyelids due to atropine sulfate used by an ophthalmologist.

Except for this woman (in whom it was considered unwise to risk the excessively severe reaction which might have occurred), all patients who developed sensitivity to Thephorin by topical application were given the drug by mouth after the acute flare had subsided. The dosage was 25 mg. 3 times daily for 1 day, unless an eruption appeared before the third dose had been taken. Six of the 11 patients so tested developed a patchy, generalized, maculopapular eruption, with a flare of all previously involved areas, at intervals ranging from ½ hour after the first to 4 hours after the third dose. The nature of the eruption indicated that there was dermal as well as epidermal sensitization to Thephorin. However, the remaining 5 subjects failed to flare after ingestion of 3 25 mg. tablets of Thephorin.

CONTROL STUDIES WITH THEPHORIN OINTMENT AND NONMEDICATED OINTMENT BASE

In 32 of the 200 patients studied, the areas of chronic lichenified dermatitis involved both sides of the body. The intensity of the bilateral eruptions was approximately equal in the individual cases. These 32 patients applied Thephorin Ointment to the lesion on the right side and Carbowax 1500 to the symmetrically involved area on the left side. There was definite improvement of lichenification and fewer excoriations on the side treated with Thephorin Ointment in 26, or 81 per cent, of these cases with no or very little objective improvement on the side treated with the nonmedicated ointment base. Three patients, or 9.5 per cent, showed no improvement on either side, and, in the remaining 3, the side treated with placebo salve showed more marked improve-

ment than the side to which Thephorin Ointment had been applied. The following case history is illustrative of the observations made in over 80 per cent of the 32 patients studied.

CASE REPORT

M. M., a 49-year-old housewife, had symmetrically distributed patches of lichen simplex chronicus (localized neurodermatitis) involving the flexor aspect of the wrists for 10 or more years. Various topical remedies including tar ointment, boric acid ointment with 0.5 per cent phenol, and ointments containing menthol, camphor and ethyl aminobenzoate had been used with little relief.

Fig. 2.—Hands and wrists of patient with symmetrically distributed patches of lichen simplex chronicus of 10 years' duration involving chiefly the wrists. Results after 1 month of treatment with Thephorin Ointment applied to the right wrist, placebo ointment to the left wrist. Note definite diminution of lichenification and excoriations on the right wrist.

Thephorin Ointment in carbowax was applied twice daily to the right wrist, carbowax alone to the left. At the end of 1 month, more than 75 per cent of the lichenification had disappeared from the right wrist, the left wrist remaining unchanged. (Fig. 2.) Improvement was maintained while Thephorin Ointment was applied over a period of about 10 weeks, but relapse occurred when Thephorin Ointment was discontinued for 2 weeks. Application of Thephorin Ointment to the left wrist resulted in more than 50 per cent objective improvement following 1 month of treatment, but relapse again occurred when the ointment was discontinued for 2 weeks.

TRIALS WITH OTHER TOPICAL MEDICATION FORMS OF THEPHORIN AND WITH NU-1525

Thephorin Lotion was tried in 80 patients with widespread pruritic dermatoses. The antipruritic effect was similar to that obtained with Thephorin Ointment. This liquid preparation also produced intense burning of acutely inflamed and abraded surfaces in the majority of cases with such acute lesions. However, patients with acute urticaria tolerated the lotion well and generally preferred it to the ointment for esthetic reasons.

Thephorin in a base containing Aquaphor and cold cream was tried in 22 patients with atopic dermatitis and xerotic skin. Objective results with this and the regular Thephorin Ointment were precisely the same, but generally these patients preferred the former preparation because it gave them greater relief from dryness. On the other hand, patients whose skin was not excessively dry gave preference to Thephorin in the carbowax base since it was more readily wiped off and did not stain.

Seventy-two patients from the groups with localized and disseminated neurodermatitis and with anogenital pruritus, who had all derived substantial improvement from Thephorin Ointment, were treated with an ointment containing 5 per cent of NU-1525 hydrobromide in a carbowax base. Later in the course of the experiment, they were handed an ointment consisting of non-medicated carbowax with the instruction to apply this to their lesions. Fifty-eight of the patients, or 80 per cent, reported relief from their itching when using NU-1525 Ointment, but stated that the third preparation (carbowax) had no antipruritic effect. The lesions of 15 of these patients involved both sides of the body. When they were tested by the method of paired symmetrical comparison using NU-1525 Ointment on one side and carbowax on the other, none of these subjects showed any objective improvement on the side treated with NU-1525.

DISCUSSION

A deliberate attempt was made to sensitize as many subjects as possible by having 200 patients with various pruritic dermatoses apply Thephorin Ointment for a prolonged period of time. It was felt that such a use test, carried out on abnormal skin, would yield more reliable data on the sensitizing capacity of Thephorin Ointment than the sensitizing index test as suggested by Sulzberger and Simon,[12] Rostenberg and Sulzberger,[13] or the similar prophetic patch test as later suggested by Schwartz and Peck,[14] since these procedures do not duplicate the conditions of actual clinical use. The minimum period of application (21 days) corresponds to the usual maximum incubation period,[15] for the development of eczematous sensitization. In many cases treatment was continued for periods of 6 months or more since the refractory period[16] preceding the sensitizing exposure[17] is variable and unpredictable. Obviously, the longer the period of clinical exposure the greater the likelihood for potential reactors to become sensitized.

This question arises. Can the incidence of induced eczematous contact-type dermatitis, as encountered in the 200 patients so treated, be taken as the true sensitizing index of Thephorin? Probably not.

Undoubtedly, the greater the case material that is being followed, the more closely the incidence of sensitization observed will reflect the true sensitizing index of any remedy. In order to arrive at a figure representing more accurately the true index of sensitization of Thephorin Ointment than the results of any individual investigator, it is permissible, we feel, to pool all the reports containing information on the sensitizing properties of this preparation.

Table III listing the pertinent findings of 8 groups of investigators, including our own, shows that of the total of 1,343 cases studied 32 patients, or 2.4 per

cent, developed true sensitization to Thephorin Ointment. This figure must be accepted as approximating the true incidence of sensitization until such time as different data on studies in far larger groups of patients become available.

TABLE III. INCIDENCE OF SENSITIZATION WITH THEPHORIN OINTMENT AS OBSERVED BY EIGHT GROUPS OF INVESTIGATORS IN A TOTAL OF 1,343 PATIENTS STUDIED

INVESTIGATOR	NUMBER OF PATIENTS TREATED	NUMBER OF PATIENTS SENSITIZED
Shelmire[2]	455	6
Ellis and Bundick[5]	50	3
Wooldridge and Joseph[6]	43	1
Flower[7]	36	0
Laymon, Madden, and Schmid[8]	324	5
D'Avanzo[9]	74	4
Reiss and Kern[10]	161	1
Present study	200	12
Totals	1,343	32 (2.4%)

These over-all findings indicate that Thephorin is a much less potent sensitizer than some of the other topically applied medications. For instance, Hopkins and Lawrence[18] reported a sensitizing index of from 11 to 25 per cent for topically applied penicillin. According to Sulzberger et al.,[19] the index of sensitization due to local sulfonamide therapy of experimental burns in man is 19 per cent. Downing et al.[20] found that 10 per cent of their patients treated with Furacin Ointment became sensitized, and Lynch[21] noted that 15 to 25 per cent of his patients developed sensitization after local administration of Furacin.

Studies into the sensitizing index of other topically applied antihistaminic drugs were not undertaken on nearly as large a scale as those reported on Thephorin Ointment. Philip[23] saw no evidence of sensitization in 85 patients treated with Benadryl Cream, and Sulzberger et al.[22] found that 2 of 90 patients applying Pyribenzamine Ointment developed "an allergy of eczematous contact-type" to the drug, an incidence of 2.2 per cent.

It is not surprising that there was no cross sensitization to Benadryl, Pyribenzamine, Trimeton, or Histadyl in any of the 12 patients of our series who became sensitized to Thephorin, since the chemical structures of the former 4 antihistaminics and of Thephorin are so widely divergent. Similarly, 1 patient who had developed sensitivity to Pyribenzamine and whom we had the opportunity of testing was not sensitive to Thephorin.

The observation that the reactions of all the 12 patients developing sensitization to Thephorin were less severe to NU-1525 is of academic interest only, since NU-1525 has little or no value in the treatment of pruritic dermatoses.

There can be no doubt that the flares developing in the 4 patients with atopic dermatitis, the 8 cases with acute eczematous contact-type dermatitis, and the 2 subjects with stasis dermatitis were not caused by Thephorin, since even use tests done at a later time were not productive of any reactions. Moreover, spontaneous flares are commonly seen in patients with eczematous dermatoses and, according to Sulzberger,[24] may be explained by alterations in sensitivity. The high incidence of flares in the patients with a diagnosis of acute eczematous contact-type dermatitis bears out Shelmire's[2] statement that Thephorin Ointment is contraindicated in acute dermatitides.

For the comparative evaluation of Thephorin Ointment and its nonmedicated base, the method of *paired symmetrical comparison* of Sulzberger et al.[25] was employed. The study of the response of 32 patients with bilateral lesions showed clearly that the clinical improvement in over 80 per cent of these cases was attributable to Thephorin and not to the carbowax base. Only objective criteria such as changes in the degree of lichenification or excoriation were used in assessing the relative merits of the ointment preparations tested, since an evaluation on the basis of subjective symptoms such as itching was found entirely misleading. The experiments with NU-1525 illustrate this very clearly. None of the 15 patients with bilateral lesions in whom the method of paired symmetrical comparison was employed showed any objective improvement on the side treated with NU-1525. This renders unreliable their own verdict that this congener of Thephorin, in contrast to carbowax, had relieved their pruritus, since any diminution of itching should have become evident by a reduction of lichenification and excoriations. Thus, the method of paired symmetrical comparison proves conclusively the merits of one topical preparation versus another.

Undoubtedly, Thephorin is distinguished by marked antipruritic action, but NU-1525 has no demonstrable antipruritic properties. Since both these substances are local anesthetics, it can be inferred that Thephorin does not relieve pruritus by virtue of its local anesthetic effect only. However, we do not proffer any discussion of the mechanism of its antipruritic action since this would lead into the realm of speculation rather than into that of interpretation of clinical experimentation.

What is the practical significance of the findings presented in this paper?

Thephorin Ointment is a valuable agent in relieving severe pruritus in a high percentage of patients, but at the same time, it is a sensitizer as all topically active compounds are. However, the sensitizing index of Thephorin Ointment is by no means so high as to exclude it from our armamentarium. On the contrary, if used judiciously, it will relieve a great number of patients suffering from pruritic dermatoses. It is imperative, though, that the rank and file of the medical profession, as well as the lay public, become aware of the great importance of discontinuing topical antihistaminic therapy—and for that matter any topical therapy—at the first sign of a flare or spread of the eruption. This lesson needs to be repeated time and again and cannot be overemphasized.

SUMMARY AND CONCLUSIONS

Two hundred patients with various pruritic dermatoses were treated with Thephorin Ointment for periods varying from 3 weeks to 6 months. One hundred thirty-five of these, or 67.5 per cent, derived benefit from the ointment; 12 patients, or 6 per cent, developed true eczematous sensitization, while 6 of these, or 3 per cent, developed dermal sensitization as well. When the more than 1,300 recorded cases treated topically with Thephorin are analyzed, an index of sensitization of the order of 2.4 per cent is obtained.

By applying the method of paired symmetrical comparison, it was demonstrated conclusively that the relief obtained from Thephorin Ointment in these cases is due to the antihistaminic agent and not to the ointment base.

Similarly, evidence is presented that the application of an ointment containing a congener of Thephorin, NU-1525, is not productive of the improvement which resulted from the use of Thephorin Ointment on the symmetrically involved sites. Since Thephorin and NU-1525 are both powerful local anesthetics, it is concluded that the antipruritic effect of the former cannot be attributed solely to its local anesthetic effect.

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American Academy of Allergy

REPORT OF THE POLLEN SURVEY COMMITTEE OF THE AMERICAN ACADEMY OF ALLERGY FOR THE SEASON OF 1949

OREN C. DURHAM, CHAIRMAN

During the past season the following persons served on the Steering Committee: Drs. A. Orville Dahl, Oren C. Durham, Theodore L. Squier, and Matthew Walzer. All allergists, botanists, public health officials, and others collaborating in carrying out and reporting local pollen surveys are regarded as members of the Committee. On this basis the total active membership during the past year numbered 71. The names of these members, together with those who reported data for the 1946-1948 seasons, appear on p. 454 where they are keyed with the data from each locality.

COVERAGE

For the 1949 season, 194 local studies have been reported in part or in total, 93 of which are for local areas not previously surveyed. During the past 4 years the Committee has carried on atmospheric pollen research in a total of 229 different localities in 7 states, the District of Columbia, 3 Canadian provinces, Mexico, and Cuba. Of the 194 local studies made during the past year, 145 were concerned only with ragweed, 15 were complete differential counts, 6 were complete for summer and fall pollens, and 28 complete for the fall season only.

THE RAGWEED SEASON

In most of the communities the ragweed figures were higher than in 1948 and higher than the average for the 3 previous years. The lowest count recorded was at Portland, Ore., where only 6 pollen grains were found through the season. The Key West, Fla., record was almost as good. There, the total over a pollinating period of at least 6 months was only 20. These two cities may thus boast the best ragweed index* figures of any sizable community in continental North America where studies have been made since 1946. Better records than these have been obtained only in some of the western national parks. However, there are zero ragweed records, obtained some years ago, and still unchallenged, for Fairbanks, Nome, Juneau, Alaska, and for Seattle, Wash. Very low ragweed counts for 1949 were turned in for Pasadena, Los Angeles, and Monterey, for Miami Beach, for several communities in northern New Hampshire, and for two towns at the end of the Gaspé Peninsula.

The apex of atmospheric contamination occurred in late May in Miami Beach and in late September in Dallas, Tex., but in almost every city in the central, northern, and eastern parts of the United States and in southern Canada the maximum concentration was noted between August 27 and September 7.

MULTIPLE RAGWEED STUDIES

In several metropolitan areas, slide exposures were made in different sections of the city and its suburbs, the counting being carried on or controlled from one laboratory. The 12 coordinated studies made in New York City and adjacent parts of Long Island and New Jersey showed considerable variation in the degree of air contamination but remarkable consistency in the date of seasonal apex—11 out of 12 falling on September 1. The 9 Philadelphia[31] stations did not agree so well. While the average apex occurred on the the twenty-ninth, it was so recorded in only 3 of the stations, 4 waiting until September 7. Exposures were made in 4 different places in Columbus, Ohio.[42]

*The index figure is an arbitrary rating based on 3 factors: average length of season, maximum concentration, and average seasonal total. (Durham, O. C.: Evaluation of the Ragweed Hay Fever Resort Areas of North America, J. ALLERGY 8: 175, 1937.)

TABLE I

RAGWEED DATA FROM THE NATIONAL POLLEN SURVEY

STATE AND CITY	POLLEN GRAINS PER CU. YD. OF AIR — ANNUAL TOTALS 1946	1947	1948	1949	MAXIMUM COUNT 1949	APEX DATE 1949	AVERAGE RAGWEED INDEX
Arizona							
Grand Canyon National Park[1]							
North Rim (fall only)	----	----	20	----	----	----	0.15
South Rim (fall only)	----	16	----	----	----	----	0.12
Phoenix (fall only)[2]	----	29	----	----	----	----	0.21
Arkansas							
Little Rock[3]	----	3,450	4,679	----	----	----	63.0
California							
Lassen Volcanic National Park[1]	----	3	----	----	----	----	0.03
Los Angeles[4]	----	60	119	62	1	9/22	0.51
Monterey[64]	----	----	----	46	1	6/4	0.24
Pasadena[5]	----	122	----	68	9	11/14	0.87
Sequoia National Park[1]	----	3	----	----	----	----	0.03
Yosemite National Park[1]	----	50	----	----	----	----	0.29
Colorado							
Colorado Springs[6]	----	1,324	1,104	----	----	----	27.0
Glenwood Springs[1]	----	----	----	68	14	8/25	0.78
Mesa Verde National Park[1]	----	----	97	----	----	----	0.52
Connecticut							
Bridgeport[7]	----	----	1,633	1,724	181	9/6	31.0
Fairfield[7]	----	----	1,391	----	----	----	26.0
Hartford[8]	----	----	3,321	3,159	510	9/2	54.0
New Haven[9]	1,906	856	1,354	876	97	9/7	23.0
Stratford[7]	----	----	1,222	----	----	----	26.0
Waterbury[10]	389	1,048	781	1,181	112	9/1	19.0
District of Columbia							
Washington[11]	2,158	2,108	2,330	2,186	220	9/1	39.0
Washington Airport[11]	----	----	2,154	----	----	----	35.0
Florida							
Everglades National Park[1]	----	----	----	----	----	----	4.0
Key West[1]	----	----	----	20	2	----	0.12
Miami Beach[12]	----	----	88	194	7	5/28	0.86
Orlando[13]	----	----	----	471	36	9/28	6.0
Georgia							
Atlanta[14]	----	1,420	1,584	----	----	----	30.0
Illinois							
Chicago[15]	4,116	7,526	4,017	8,067	583	9/4	70.0
Decatur[16]	----	9,660	----	----	----	----	114.0
Peoria[17]	----	13,071	10,324	----	----	----	108.0
Rockford[18]	----	9,471	6,569	11,083	814	8/30	91.0
Indiana							
Evansville[19]	----	----	----	12,356	947	9/7	111.0
Indianapolis[20]	----	6,655	7,591	13,633	1,102	8/24	121.0
Iowa							
Iowa City[21]	10,178	13,046	8,851	4,626	415	8/31	97.0
Kansas							
Goodland[1]	----	731	----	----	----	----	23.0
Kentucky							
Trappist[22]	----	----	----	7,846	885	9/5	86.0

In the Montreal metropolitan area 4 individuals made independent pollen studies in a total of 21 different locations (Mitchell,[1] Rose,[1] Cabana,[1] Campagna[10]). All 4 agreed on the apex date, August 27, while the ragweed index ratings ranged from 32 to 51.

TABLE I—CONT'D

STATE AND CITY	POLLEN GRAINS PER CU. YD. OF AIR — ANNUAL TOTALS 1946	1947	1948	1949	MAXIMUM COUNT 1949	APEX DATE 1949	AVERAGE RAGWEED INDEX
Maine							
Alfred[23]	----	----	----	1,534	252	9/4	23.0
Bar Harbor[23]	----	----	----	320	90	8/28	6.0
Boothbay Harbor[23]	----	----	----	320	68	8/27	5.0
Greenville Junction[23]	----	----	----	115	32	8/26	2.0
Jackman[23]	----	----	----	479	101	8/27	9.0
Lincoln[23]	----	----	----	94	47	8/28	2.0
Newport[23]	----	----	----	389	65	9/7	10.0
New Portland[23]	----	----	----	137	22	8/27	1.0
North Augusta[23]	----	----	----	918	122	8/28	18.0
Oquossoc[23]	----	----	----	169	47	9/1	3.0
Orono[23]	----	----	----	587	166	8/28	13.0
Portland[23]	----	----	----	1,469	158	8/28	24.0
Maryland							
Bethesda[11]	----	----	5,755	----	----	----	65.0
Michigan							
Ann Arbor[24]	----	13,758	12,258	11,002	1,116	9/2	116.0
Detroit[25]	----	4,989	3,067	5,144	417	8/27	66.0
Minnesota							
Minneapolis[26]	----	----	----	24,473	2,574	8/24	200.0
Missouri							
St. Louis[27]	----	----	----	13,489	1,112	8/19	124.0
Montana							
Glacier National Park[1]							
Belton	11	----	----	----	----	----	0.10
Many Glacier	9	----	----	----	----	----	0.07
Nebraska							
Omaha[28]	----	12,207	9,307	----	----	----	90.0
Nevada							
Lake Mead[1]	----	389	----	----	----	----	4.0
New Hampshire[29]							
Bath	----	----	185	169	54	8/28	3.0
Berlin	----	274	1,119	288	32	9/1	11.0
Bethlehem	----	----	----	209	47	8/28	6.0
Carrol	----	----	----	54	14	8/26	0.41
Charlestown	----	----	540	828	241	9/2	14.0
Colebrook	----	15	79	61	22	9/7	2.0
Concord	----	264	208	500	90	8/27	7.0
Conway	----	----	----	299	54	8/26	6.0
Dixville	----	----	----	126	36	9/5	3.0
Errol	----	----	46	112	22	8/27	0.62
Exeter	----	329	2,069	1,278	256	9/2	17.0
Groveton	----	----	135	245	47	9/4	2.0
Hillsboro	----	----	189	331	122	8/29	5.0
Hinsdale	----	----	328	738	86	9/5	11.0
Holderness	----	----	----	230	47	8/28	5.0
Keene	----	220	585	382	47	9/3	8.0
Laconia	----	29	1,073	198	58	9/1	12.0
Lancaster	----	----	----	115	18	9/1	0.75
Lebanon	----	4	1,797	918	176	8/30	17.0
Lincoln	----	----	217	198	61	8/27	3.0
Littleton	----	----	----	86	22	8/28	2.0
Manchester	----	----	501	644	101	8/28	11.0

DIFFERENTIAL COUNTING

Special attention is called to the condensed records in Table II where all types of pollen were counted over periods of 4 to 12 months. The ideal procedure is to identify and count everything in the air during the whole season of local air contamination. In the table given here no account is taken of pine or closely related conifer pollens except in the case of deodar cedar at Pasadena, Calif.

TABLE I—CONT'D

STATE AND CITY	POLLEN GRAINS PER CU. YD. OF AIR — ANNUAL TOTALS 1946	1947	1948	1949	MAXIMUM COUNT 1949	APEX DATE 1949	AVERAGE RAGWEED INDEX
New Hampshire—Cont'd							
Moosilaukee	----	----	----	68	11	9/1	0.45
Nashua	----	137	2,542	2,927	464	9/2	28.0
New Ipswich	----	----	360	580	148	8/19	9.0
New London	----	0	177	677	140	9/1	6.0
North Conway	----	32	300	----	----	----	4.0
Ossipee	----	----	112	173	32	8/28	3.0
Pittsburg	----	----	87	191	32	8/27	3.0
Plymouth	----	----	201	----	----	----	4.0
Rochester	----	----	809	788	79	9/4	17.0
Rye	----	----	590	810	83	9/3	15.0
Warren	----	47	137	194	58	9/7	2.0
Weirs	----	----	----	371	50	9/11	9.0
Whitefield	----	----	90	230	29	8/27	2.0
New Jersey							
Dover[30]	----	----	1,167	----	----	----	21.0
Freehold[30]	----	----	6,126	----	----	----	72.0
Maplewood[30]	----	1,998	1,003	1,129	161	9/1	21.0
Marlboro[30]	----	----	----	3,129	473	9/1	40.0
New Brunswick[30]	----	830	7,036	4,360	607	9/1	74.0
Pitman[31]	----	3,995	----	----	----	----	51.0
Sandy Hook[30]	----	----	1,719	----	----	----	39.0
Teaneck[30]	----	1,809	----	----	----	----	31.0
Verona[30]	944	1,306	1,710	2,180	278	9/1	33.0
Westwood[30]	----	----	839	----	----	----	18.0
New Mexico							
Albuquerque[32]	----	97	674	792	90	----	9.0
New York							
Albany[33]	2,250	2,374	2,282	4,586	366	----	45.0
Ausable Forks[33]	----	----	872	----	----	----	20.0
Big Indian[33]	----	----	----	216	36	----	3.0
Big Moose[33]	260	242	296	510	68	----	6.0
Bronx[30]	1,203	1,121	----	1,082	196	9/1	25.0
Brooklyn[30]	853	1,043	----	1,258	331	9/1	25.0
Buffalo[34]	4,994	4,576	3,261	3,522	396	9/4	56.0
Croton[30]	1,163	1,728	----	----	----	----	29.0
East Berne[33]	----	----	1,604	----	----	----	32.0
Elsmere[33]	----	----	----	----	----	----	49.0
Fire Island[33]	----	1,072	----	----	----	----	23.0
Fleischmanns[33]	----	----	----	466	124	----	7.0
Flushing[35]	900	1,865	1,285	1,934	266	9/1	29.0
Garden City[36]	----	1,198	1,437	1,998	458	9/1	31.0
Hague[33]	856	612	80	774	480	----	16.0
Haines Falls[33]	----	----	----	----	----	----	5.0
Hunter[33]	----	----	572	3,464	370	----	29.0
Indian Lake[33]	----	358	226	630	80	----	7.0
Jamaica[30]	----	1,596	----	----	----	----	35.0
Jamestown[33]	5,616	----	----	----	----	----	64.0
Keene[33]	----	----	----	444	56	----	8.0
Keene Valley[33]	----	78	158	254	32	----	1.0
Keesville[33]	----	----	----	1,380	114	----	26.0
Lake George[33]	----	----	----	1,238	148	----	23.0
Lake Kushaqua[33]	----	----	----	982	144	----	18 0
Lake Placid[33]	292	----	414	764	104	----	10.0
Liberty[33]	----	----	----	1,310	128	----	25.0
Long Lake[33]	206	218	278	390	68	----	4.0
Loon Lake[33]	----	----	204	----	----	----	5.0
Lowville[33]	----	----	----	--	----	----	20.0

TABLE I—CONT'D

STATE AND CITY	POLLEN GRAINS PER CU. YD. OF AIR — ANNUAL TOTALS 1946	1947	1948	1949	MAXIMUM COUNT 1949	APEX DATE 1949	AVERAGE RAGWEED INDEX
New York—Cont'd							
Manhattan[30]	1,327	1,808	----	1,497	205	9/4	26.0
McKeever[33]	----	126	616	908	106	----	10.0
Minnewaska[33]	----	----	----	777	282	----	22.0
New York Metropolitan Ave.[30]	1,217	1,564	1,911	1,991	360	9/1	34.0
North Creek[33]	1,164	968	630	1,654	146	----	20.0
Northville[33]	----	----	746	2,250	332	----	27.0
Old Forge[33]	----	----	----	704	112	----	16.0
Paul Smiths[33]	----	----	300	308	74	----	6.0
Phoenicia[33]	----	----	2,116	388	34	----	20.0
Pine Hill[33]	340	396	378	236	28	----	6.0
Plattsburg[33]	1,934	2,636	----	----	----	----	41.0
Pomona[33]	----	----	----	1,770	172	----	30.0
Port Henry[33]	----	----	746	----	----	----	14.0
Raquette Lake[33]	----	----	----	526	94	----	9.0
Remsen[33]	956	23,362	1,202	1,974	236	----	40.0
Rochester[37]	2,172	2,396	1,558	5,725	593	8/30	47.0
Rockaway[30]	----	1,515	----	3,274	800	9/1	43.0
Saranac Lake[33]	----	----	802	1,138	170	----	19.0
Shroon Lake (Severence)[33]	----	290	154	448	54	----	6.0
Speculator[33]	790	68	366	850	114	----	9.0
Staten Island[30]	1,350	1,906	----	2,366	321	9/1	36.0
Tannersville[33]	356	294	324	----	----	----	5.0
Tupper Lake[33]	----	428	352	602	58	----	8.0
Wanakena[33]	376	404	278	692	58	----	7.0
White Plains[30]	----	1,090	1,426	1,425	244	9/1	27.0
Windham[33]	1,722	46	2,782	1,880	180	----	28.0
Zena[33]	----	484	788	610	78	----	12.0
North Carolina							
Charlotte[38]	----	3,592	1,975	2,168	198	9/1	44.0
Ohio							
Akron[39]	10,011	8,772	7,030	12,538	1,317	9/5	99.0
Cincinnati[40]	----	----	----	12,134	1,181	9/2	109.0
Cleveland[41]	5,483	4,035	5,385	6,599	457	9/1	66.0
Columbus[42]	----	----	----	6,910	685	8/28	75.0
Dayton[43]	----	----	----	9,361	857	9/4	92.0
Toledo[44]	11,475	14,307	12,613	19,460	1,570	8/27	136.0
Youngstown[45]	4,741	5,063	4,511	7,211	738	9/2	69.0
Oregon							
Crater Lake National Park[1]	----	7	----	----	----	----	0.07
Portland[46]	----	----	----	6	1	----	0.05
Pennsylvania							
Broomal[31]	----	2,208	----	----	----	----	40.0
Hatboro[31]	----	3,560	----	----	----	----	48.0
McKeesport[47]	----	----	----	12,455	928	9/1	117.0
Meadville[48]	----	----	----	5,690	460	9/2	70.0
Philadelphia[31]	3,830	2,549	3,177	4,036	343	8/29	54.0
Pittsburgh[49]	6,600	3,449	7,619	17,328	1,249	9/2	93.0
Rhode Island							
Providence[50]	----	1,162	1,045	1,421	144	9/1	28.0
Tennessee							
Great Smoky Mts. National Park[1]							
Headquarters	----	582	----	----	----	----	13.0
Newfound Gap	----	204	----	----	----	----	4.0
Nashville[51]	9,470	4,488	6,168	7,213	688	9/6	70.0

TABLE I—CONT'D

STATE AND CITY	POLLEN GRAINS PER CU. YD. OF AIR — ANNUAL TOTALS 1946	1947	1948	1949	MAXIMUM COUNT 1949	APEX DATE 1949	AVERAGE RAGWEED INDEX
Texas							
Dallas[52]	13,523	21,810	25,543	20,338	832	9/23	154.0
Utah							
Bryce Canyon National Park[1]	----	----	148	----	----	----	0.88
Salt Lake City[53]	----	913	459	473	40	9/9	10.0
Zion National Park[1]	----	56	169	----	----	----	0.68
Vermont							
Burlington[54]	----	----	3,700	2,264	131	9/4	49.0
Virginia							
Shenandoah National Park[1]							
Headquarters	----	----	2,165	----	----	----	35.0
Big Meadows	----	----	638	----	----	----	10.0
Washington							
Olympic National Park[1]							
Headquarters (Elwha)	----	4	7	----	----	----	0.09
Hurricane Ridge	----	----	7	----	----	----	0.09
Mount Rainier National Park[1]							
Longmire	----	0	----	----	----	----	0
Paradise	----	12	----	----	----	----	0.1
White River	----	4	----	----	----	----	0.04
Wisconsin							
Madison[55]	4,620	14,946	4,725	5,387	620	8/28	87.0
Milwaukee[56]	9,916	11,656	6,688	3,678	1,656	9/1	93.0
Wyoming							
Grand Teton National Park[1]	----	----	14	----	----	----	0.11
Manitoba							
Winnipeg[57]	----	492	701	274	50	8/21	8.0
Ontario							
Hamilton[58]	5,772	12,618	4,688	6,876	1,109	9/5	84.0
Quebec							
Carleton (Bonaventure)[65]	----	----	----	76	29	8/31	2.0
Gaspé[65]	----	----	----	58	22	9/5	0.51
Grande-Rivière (Gaspé-Sud)[65]	----	----	----	40	14	8/31	0.24
Lac des Seize-Iles (Argenteuil)[65]	----	----	----	702	115	9/4	15.0
Limoilou (Quebec)[65]	----	----	----	846	75	9/3	18.0
Montreal (by Mitchell)[59]	----	----	1,353	3,086	274	8/27	32.0
Montreal (by Cabana)[60]	----	----	----	2,537	288	8/27	40.0
Montreal (by Rose)[61]	----	----	----	3,323	413	8/27	49.0
Montreal (by Campagna)[65]	----	----	----	3,450	337	8/27	51.0
Percé (Gaspé-Sud)[65]	----	----	----	47	29	8/31	2.0
Rivière-du-Loup[65]	----	----	----	490	97	9/4	8.0
Rimouski[65]	----	----	----	184	68	9/4	3.0
Ste-Anne-de-la-Pocatière (Kamouraska)[65]	----	----	----	547	104	8/26	16.0
Cuba							
Havana[62]	----	30	----	----	----	----	0.18
Mexico							
Mexico City[63]	----	----	745	568	36	9/13	14.0

TABLE II. POLLEN RECORDS FOR 1949 IN POLLEN GRAINS PER CUBIC YARD
(Monthly Totals)

POLLEN	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL
Los Angeles,[4] Calif.													
Alder	11	11											22
Elm		3	6										9
Walnut		2	2	72	9	1							86
Cypress		10	30	28	8								76
Acacia		3	6	1			2				4		16
Sycamore			18	18									36
Oak			2	62	10								74
Olive				9	99	36							144
Grass	4	2	12	16	62	36	24	20	26	16	12	2	232
Chenopodium			2	3	10	12	15	19	12	9	3		85
Sage	4						4	14	16	24	8		66
Ragweed				9	7	2	3	7	18	11	5		62
Pasadena,[5] Calif.													
Oak			9	268	75	11	1						364
Olive				2	158	108	6						274
Walnut			4	90	22	7	4						127
Cottonwood			14										14
Elm			8						64	36	3		111
Sycamore				108	8								116
Eucalyptus	10	3	10	54	2	15				2	3	7	106
Alder	25	15	15										55
Acacia	1	7	9	15	1	8		2					43
Deodar									3	98	9		110
Palm				5		3				8		3	19
Grass	10	3	36	172	134	110	84	65	36	37	60	25	772
Chenopodium			4	10	4	12	16	55	15	15			131
Plantain							1	3					4
Sage	5	1					1	14	9	27	29	16	102
Ragweed							2	4	28	21	13		68
Miscellaneous	28	65	161	189	184	154	72	74	93	77	69	46	1,212
Glenwood Springs,[1] Colo. (June-September)													
Oak						54							54
Cedar						3							3
Plantain						12							12
Grass						90	12	2					104
Russian Thistle						2	5	13					20

Kochia								3	3				6
Other Chenopods						16		8	2				26
Sagebrush								13	192				205
Composite									4				4
Ragweed						3		50	18				71
Miscellaneous						54	6		10				70
Washington,[11] D. C.													
Juniper	11	88	49		8								156
Elm		250	92										342
Maple		6	46	255	269	6							582
Alder		46		2									48
Poplar		2	54	90									146
Birch		6	12	116	70								204
Willow			4	50									54
Oak				1,043	16								1,059
Hickory				8	68	1							77
Beech				6	56	1							63
Ash				107									107
Paper Mulberry				2,520									2,520
Sycamore				1,241	3	6	6						1,256
Linden				8	49								57
Walnut				10	12								22
Sweet Gum				39	3	3							45
Grass				4	130	104	14	27	64	23			366
Plantain					40	138	26	24	3	4			235
Red Sorrel				3	146	20							169
Red Mulberry				42	90	3							135
Tree of Heaven					61	94							155
Composite						4		7	16	6			33
Chenopodium							2	8	22	2			34
Ragweed								610	1,537	39			2,186
Miscellaneous		4	28	650	362	108	26	16	34	11			1,239
Key West,[1] Fla. (June-November)													
Grass						20	61	63	29	49	2		224
Chenopodium						1	7	2	1	5			16
Magnolia						2		3					5
Sedge							2						2
Composite							9	1		3	1		14
Ragweed						2	5	9	4				20
Miscellaneous						9	124	8	27	11	1		180

TABLE II.—CONT'D

POLLEN	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL
Miami Beach,[12] Fla.													
Australian Pine		272	74	81	5	2							434
Grass	3	15	5	45	37	35	10	14	13	8	7	11	203
Amaranth		2			2	1	1		1				7
Ragweed					49	31	20	12	1	1	80		194
Orlando,[13] Fla. (Sept. 1, 1948—Sept. 1, 1949)													
Grass	74	46	398	358	306	166	114	130	92	44	48	56	1,8. 2
Oak	10											15	25
Alder	2	20							8				30
Magnolia									10				10
Ragweed							22	133	266	50			471
Miscellaneous									56			4	60
Albuquerque,[32] N. Mex. (February-December)													
Elm		4,817	3,460										8,277
Juniper		3	2,376	190	25								2,594
Poplar			1,735	2,690	13								4,438
Oak				46	30								76
Tamarisk				303	92								395
Grass					290	186	160	196	10				842
Russian Thistle					7	197	443	340	84	12		3	1,086
Kochia								140	109	9			258
Composite							168	188	36	12			404
Ragweed								241	421	126	4		792
Miscellaneous			28	40	110	38	16	74	54	8	2	16	386
Garden City,[26] N. Y. (March-September)													
Maple			136	56									192
Birch			2	747	250	1							1,000
Poplar			1	11									12
Ash			1	137	12								150
Sweet Gum				6	1								7
Willow				11	20								31
Elm				38									38
Beech				1	65								66
Hickory				2	2	1							5
Sycamore				29	107	7							143
Black Walnut				3	4								7

Oak				586	1,069	3							1,658
Grass				10	144	184	63	12	83				496
Plantain				1	56	75	29	18	8				187
Dock					32	67							99
Linden						5	2						7
Composite						2	1						3
Chenopodium							3	19	11				33
Ragweed								433	1,565				2,098
Miscellaneous					1								1
Charlotte,[38] N. C. (January-October)													
Cedar	162	47											209
Alder	206	9											215
Elm	75	301	16										392
Oak		8	441	1,500	107								2,056
Walnut			62	60	7								129
Hickory			118	99	1								218
Pecan			75	133	89								297
Ash				42									42
Willow				5									5
Grass			22	112	78	94	10	22	66	14			418
English Plantain					2	40	11	2	6				61
Ragweed								364	1,786	18			2,168
Portland,[46] Ore. (February-October)													
Hazel		136	236										372
Willow		14	20	32									66
Cedar		242	927	66	14								1,249
Elm			270	48	3								321
Alder			1,065	816	15	2							1,898
Birch				93	4								97
Poplar			2	40									42
Maple				78	15								93
Ash				46	23								69
Oak				2	28	2							32
English Walnut				3	48								51
Grass				28	150	486	140	40	10				854
Plantain			13		26	96	111	23	21				290
Chenopodium			15	2	16	7	27	9	14				90
Composite					2								2
Sedge						6							6
Ragweed						4	7	7	4				22

TABLE II.—CONT'D

POLLEN	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL
Sage													6
Miscellaneous				4	10	18	2	2	4	2			36
Providence,[50] R. I. (May-October)													
Birch					422								422
Oak					931	6							937
Ash					69								69
Maple					42								42
Elm					5								5
Grass					224	390	94	12	16				736
Ragweed								575	834	12			1,421
Salt Lake City,[53] Utah (April-September)													
Alder				79									79
Juniper				57									57
Poplar				612	27								639
Box Elder				1,248	148								1,396
Birch				6	4								10
Willow				22	4								26
Wingscale				20	51	79	46						196
Grass					74	94	82	16	4				270
Greasewood					15	2							17
Scrub Oak					75	20							95
English Plantain						26	19	4	2				51
Ragweed						4	14	150	305				473
Tree of Heaven						4	4						8
Russian Thistle							131	118	22				271
Kochia							2	234	28				264
Sugar Beet								70					70
Sage								48	386				434
Composite								2	2				4
Pigweed								7	10				17
Miscellaneous				18	26	34	12	28	12				130
Milwaukee,[56] Wis. (April-October)													
Willow				10									10
Maple				116									116
Box Elder				8	488								496
Elm				543	134								677

Hickory				3	34	10							47
Birch				2	23								25
Ash					31								31
Oak					258								258
Grass					10	708	194						912
Dandelion					2	2							4
Miscellaneous							2						2
Plantain								4					4
Ragweed								1,722	1,949	7			3,678
Winnipeg,[57] Manitoba (April-October)													
Alder				12									12
Poplar				48									48
Elm				905	3								908
Hazel				12									12
Maple				150	54								204
Willow				6	4								10
Ash				2	14								16
Birch					25								25
Oak					132	3		1					136
Grass						20	38	12	8				78
Mustard						19	2						21
Chenopodium							28	65	11				104
Ragweed								216	54	4			274
Sage								15	6				21
Miscellaneous					1	2	10		2				15
Mexico City,[63] Mexico (January-October)													
Ash	838	3,051	497	6						15			4,407
Cedar	426	564	347	41	104	82		47	69	176			1,756
Alder	397	391	210	33	11								1,042
Oak		91	607	125	33								856
Grass			22	46	66	152	208	218	22	12			746
Composite				18	30					46			94
Amaranth						12	2	29	12				55
Ragweed							4	194	334	36			568

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3. Alan G. Cazort
4. Abraham M. Targow
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61. Bram Rose
62. José M. Quintero
63. José L. Cortés
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REPORT OF THE SUBCOMMITTEE ON MOLD SURVEY OF THE RESEARCH COUNCIL, AMERICAN ACADEMY OF ALLERGY, FOR THE SEASON OF 1949

LEONARD H. HARRIS, M.D.,* CHAIRMAN

This report represents a summary of the 1949 activities of 38 contributors† to the Mold Survey Committee sponsored by the Research Council of the American Academy of Allergy.

TECHNIQUE

Daily exposures and mold sampling were made by either the Petri dish or glass slide technique or both. The plate technique consisted of exposing standard Petri dishes covered with casein hydrolysate agar medium for a period of 12 minutes each day. The period of study varied with the locality but was usually from April through October, or longer. The colonies were identified after maturation, totals computed and reported to the committe chairman. Dr. Clyde Christensen aided in the identification of the more troublesome species.

The slide method was in conformity with the standards set up by the pollen committee of the American Academy of Allergy as outlined in THE JOURNAL OF ALLERGY, May, 1946. Slides were exposed daily and the mold spores found on one square centimeter were recorded. By this method counts were obtained from April through October, or longer, for Alternaria, Hormodendrum, rusts, and smuts.

GEOGRAPHIC INCIDENCE OF AIR-BORNE MOLD SPORES

The accompanying map (Fig. 1) shows the geographic incidence of Alternaria, Hormodendrum, and the total of all mold spores, as found by exposing agar plates daily at 27 stations from May 1 to Nov. 1, 1949.

The incidence of Alternaria and Hormodendrum is found to be greatest in the Great Plains area, lower in the eastern states, and minimal in the South and Southwest. Relatively high Hormodendrum counts were found in the Los Angeles area.

ALTERNARIA

The total monthly Alternaria counts at 33 stations are tabulated in Table I. This table shows the monthly totals by comparative daily Petri dish and slide techniques. Eight midwestern cities (Madison, Wis., Peoria, Ill., Chicago, Ill., Indianapolis, Ind., Des Moines, Iowa, Oklahoma City, Okla., Ann Arbor, Mich., and Evanston, Ill.) were found to have total Alternaria plate counts for the 6-month summer season over 1,500. In these midwestern cities the peak months varied from June to October. This is in keeping with the previously observed unpredictable seasonal variation in the incidence of Alternaria. The accompanying graph for Madison, Wis. (Fig. 2), selected as typical for these 8 cities shows peaks for Alternaria from August through October. Seven other cities had total Alternaria counts over 1,200 for the same season. These were Johnson City, Tenn., Montreal, Canada, Toledo, Ohio, Cleveland, Ohio, Richmond, Va., Milwaukee, Wis., and Hamilton, Ontario. The Alternaria curve for Richmond, Va., is shown in Fig. 3. It will be noted that most of the 17 cities reporting low Alternaria counts, below 1,000 for the season, are in the southern and western states. The Los Angeles peak for Alternaria (Fig. 4) is found in late May and early June. The graph of Tucson, Ariz. (Fig. 5), is included to show the absence of seasonal peaks in that area.

*From the Northwestern University Medical School, Division of Allergy, Chicago, Ill.

†For the list of contributors, see pp. 463 and 464.

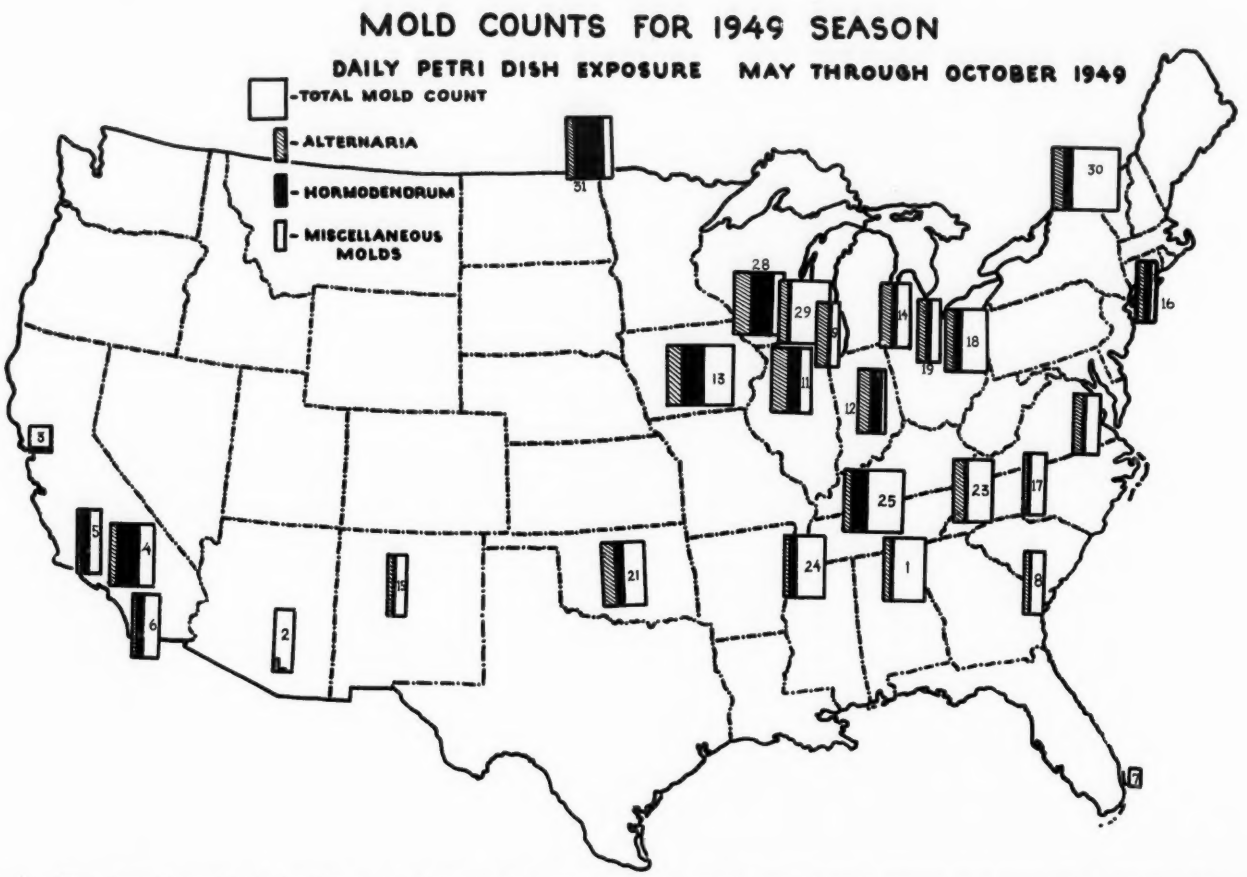

Fig. 1.—Atmospheric incidence of Alternaria, Hormodendrum, miscellaneous mold spores, and total of all molds. The area of each segment of the blocks varies with the total spores found in the particular city for 6-month period, May through October, 1949. The number in the block indicates the contributor and city (see list of contributors).

An examination of Table I will show only a fair amount of agreement in the monthly totals of Alternaria by the two methods. With the plate technique arbitrarily chosen as a standard, it was felt that the slide counts ought to agree within 25 per cent to be considered comparable. It will be seen in Table I that 19 contributors offer 101 pairs of monthly figures by both techniques for comparison. Of the 101 monthly figures 22 differed by less than 25 per cent and 79 differed by over 25 per cent so that the chances of fair agreement by both methods was not good. The only conclusion to be drawn at this time is that slide counts and plate counts for Alternaria are, by and large, in rather poor agreement. This is known to be true for Hormodendrum as well. Changes in survey techniques, it is hoped, may narrow this wide discrepancy in the future.

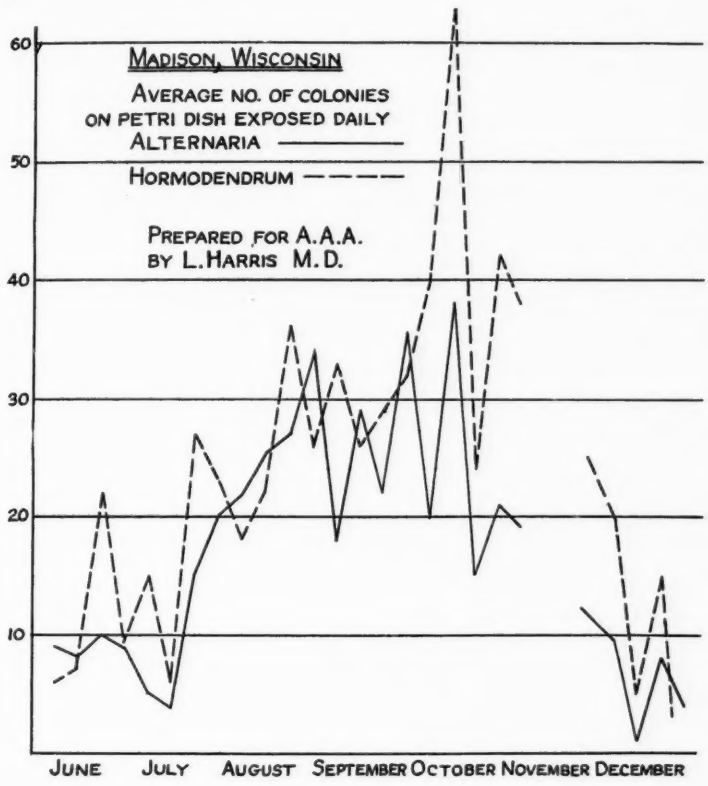

Fig. 2.

HORMODENDRUM

Table II shows the total monthly Hormodendrum colonies found on Petri dishes exposed daily at 25 stations from May to Nov. 1, 1949. The Los Angeles graph (Fig. 4) shows high counts in May and June, then again in October. Winnipeg, Canada, had peaks in June, July, and August while Madison, Wis. (Fig. 2), showed progressively increasing counts from June through October. Very high counts were found in September and October in Nashville, Tenn. Richmond, Va. (Fig. 3) and Tucson, Ariz. (Fig. 5), illustrate the nonseasonal incidence in southern cities.

STUDIES OF OTHER MOLDS

In addition to the studies of Alternaria and Hormodendrum, numerous workers made daily studies of other air-borne mold spores throughout the 1949 season.

Albuquerque, N. Mex.: Dr. Walter Werner made daily studies from April through October, 1949. He reported low counts for Aspergillus and Penicillium, moderate amounts of yeasts, Alternaria, Hormodendrum and slime molds (maximum count 131 for month of April). There was only a slight rise in counts from July through October.

Ann Arbor, Mich. (University Hospital): Daily slide and plate counts were carried out by Drs. Sheldon, Lovell, and Ludwig from April through October. Alternaria and Hormodendrum represented about 50 per cent of the mold spores in this area (Tables I and II). Relatively large counts of Aspergilli were reported on plates in April (140), July (112), and August (206). Relatively moderate amounts of Penicillium and yeasts (1 to 3 daily) were reported with little or no significant monthly variation.

Charlotte, N. C.: The figures submitted by Dr. L. C. Todd showed that Alternaria and Hormodendrum colonies comprised only about 30 per cent of all molds in his study carried out daily from Jan. 1 to Dec. 31, 1949 (Tables I and II). The remainder included low counts (1 or 2 daily) of Torula, Monilia, Penicillium Helminthosporium, and Aspergillus with occasional Rhizopus and Mucor. There were no striking seasonal fluctuations.

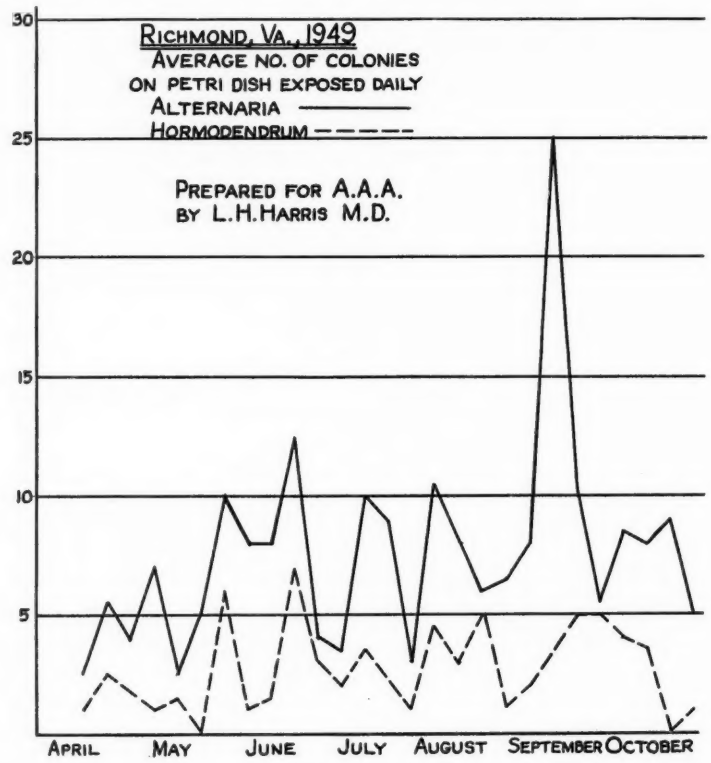

Fig. 3.

Cleveland, Ohio: The report of Dr. H. J. Friedman includes daily Petri dish and slide studies from April to Nov. 1, 1949. His figures for Alternaria and Hormodendrum showed that these comprised about one-third of the total (Tables I and II). Yeasts comprised another third of the total, with April (623) and May (536) the peak months. The remainder comprised moderate amounts of Aspergillus, Botrytis, Helminthosporium, Penicillium, and occasional Monilia and Phoma, as well as many unidentified colonies.

Des Moines, Iowa: This study by Drs. Louis J. Noun, W. E. Jordon, and H. G. Woodruff showed one of the highest total mold counts in the country in 1949. Alternaria and

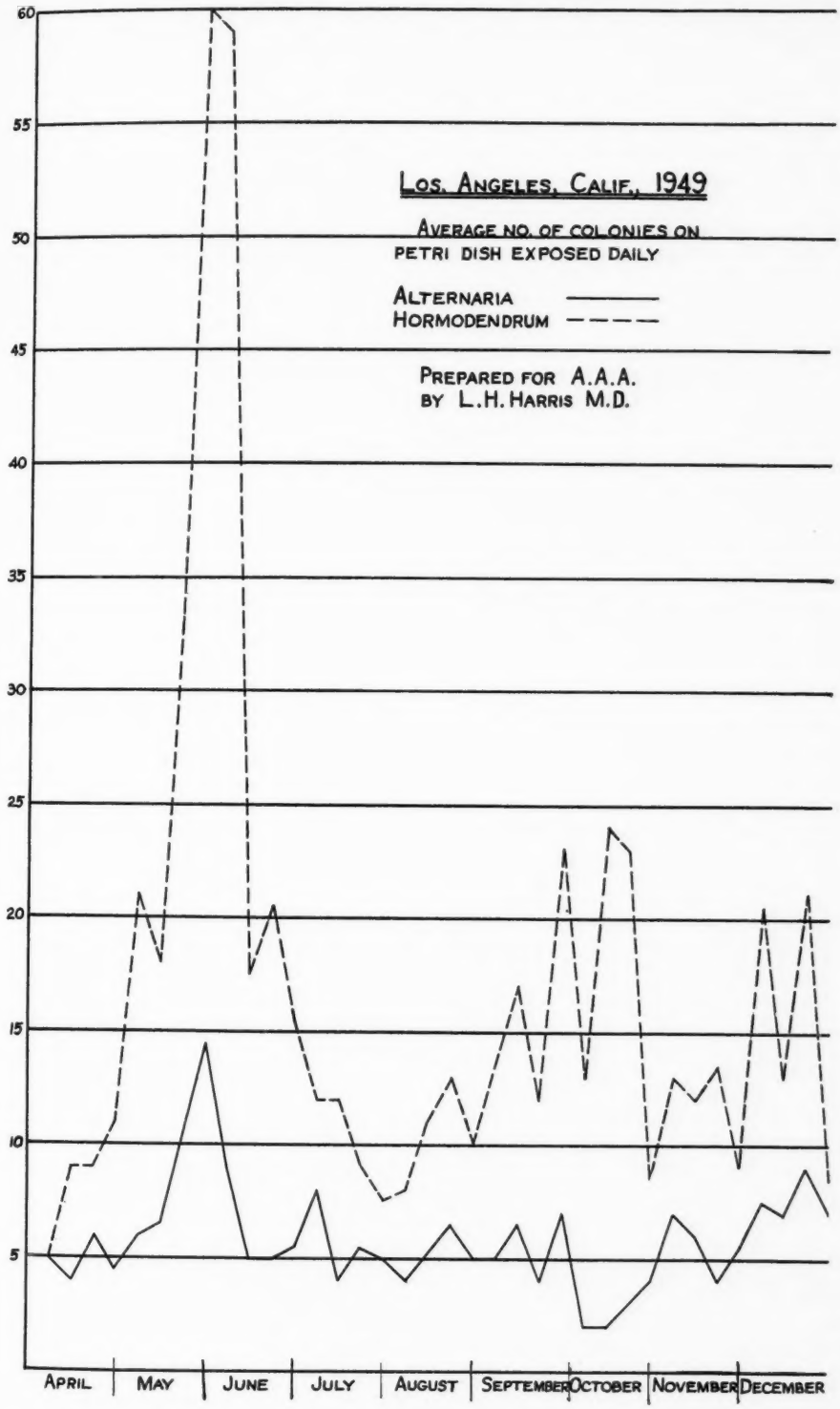
60
55
50
45
40
35
30
25
20
15
10
5
LOS. ANGELES, CALIF., 1949
AVERAGE NO. OF COLONIES ON PETRI DISH EXPOSED DAILY
ALTERNARIA
HORMODENDRUM
PREPARED FOR A.A.A.
BY L.H. HARRIS M.D.
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER

Fig. 4.

Hormodendrum comprised over 50 per cent of the total count (Tables I and II). There was a low incidence of Aspergillus, Botrytis, Monilia, Penicillium, yeast, Phoma, and Rhizopus with a very large incidence of unidentified mold colonies from April through October.

Johnson City, Tenn.: The daily plate study by Dr. C. P. Wofford showed that Alternaria and yeasts each comprised about 25 per cent of the total mold count from April to Nov. 1, 1949. Low counts (1 to 3 daily) were reported for Monilia during this period and similar amounts of Aspergillus, Penicillium, Helminthosporium, and Botrytis.

Los Angeles, Calif.: A very detailed study was undertaken by Dr. A. M. Targow by both slide and plate techniques from April to Dec. 31, 1949. Previous reference was made to the high Hormodendrum counts which comprised 50 per cent of the total seasonal incidence. Alternaria comprised 14 per cent. The remainder included a low incidence of the following with only slight seasonal variation: Actinomyces, Aspergillus, Botrytis, Cephalosporium, Chaetoconidium, Epicoccum, Fusarium, Helminthosporium, Macrosporium, Nigrosporium, Penicillium, Phoma, Pullularia, Torula, Stemphylium, and yeast.

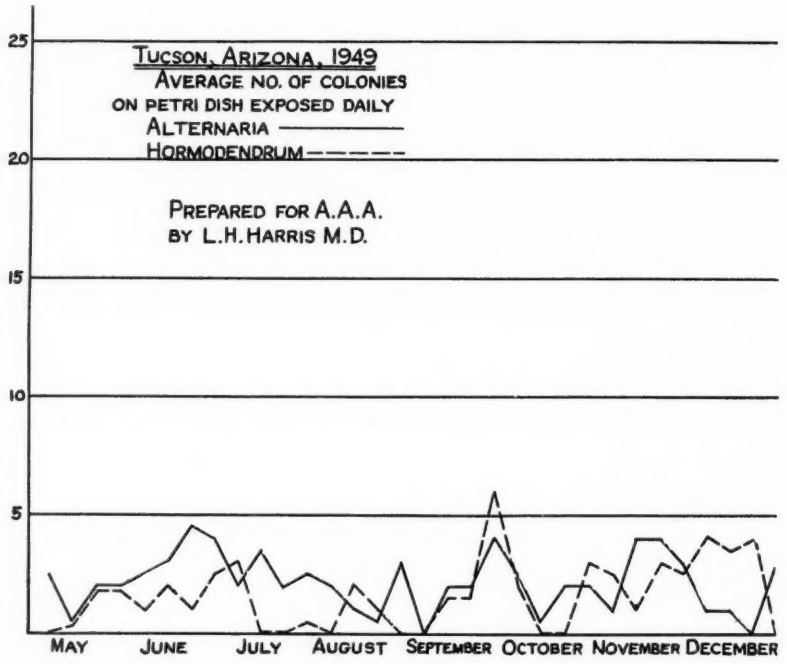

Fig. 5.

Santa Barbara, Calif.: This report by S. Allen indicated that the counts for Alternaria and Hormodendrum were less than one-half of those reported at Los Angeles.

San Diego, Calif.: A report from Dr. E. L. Keeney indicates relatively low counts for Alternaria, about 5 per cent of the total. Hormodendrum colonies comprised the highest count and were about one-third of the total. The incidence of yeasts were reported in May, June, July, and October, to vary from 30 to a peak of 115 for the month of June.

Madison, Wis.: This study by Helen Davis extended from June to Dec. 31, 1949. Alternaria and Hormodendrum counts comprised 80 per cent of the total (Tables I and II). There were low daily counts for Aspergillus, Penicillium, Mucor, yeast, and Botrytis, with no significant variation.

Miami Beach, Fla.: Dr. Nelson Zivitz reported plate counts for August, September, October, and the first half of November. While the period studied was short, the report

TABLE I. TOTAL ALTERNARIA COUNTS. DAILY SLIDE AND PETRI DISH METHODS, MAY THROUGH OCTOBER, 1949

CITY	CONTRIBUTOR NO.*	METHOD	MONTHLY ALTERNARIA COUNTS						TOTAL ALTERNARIA
			MAY	JUNE	JULY	AUG.	SEPT.	OCT.	
Madison, Wis.	28	Plate	–	309	333	745	739	710	2,836
		Slide	–	129	433	1,145	882	347	2,940
Peoria, Ill.	11	Plate	145	300	638	537	490	465	2,575
		Slide	–	–	1,262	801	435	391	2,889
Chicago, Ill.	9	Plate	–	–	472	458	567	662	2,159
		Slide	–	–	412	262	337	1,024	2,035
Indianapolis, Ind.	12	Plate	–	–	–	–	–	–	–
		Slide	34	261	604	498	459	268	2,104
Des Moines, Iowa	13	Plate	140	176	515	938	229	50	2,048
		Slide	83	305	818	405	198	–	1,809
Oklahoma City, Okla.	21	Plate	471	334	222	450	410	50	1,937
		Slide	–	–	–	–	–	–	–
Ann Arbor, Mich.	14	Plate	242	522	50	94	537	486	1,931
		Slide	87	667	1,253	984	364	1,923	5,278
Evanston, Ill.	10	Plate	–	–	–	–	–	–	–
		Slide	50	157	608	407	375	–	1,597
Johnson City, Tenn.	23	Plate	100	150	132	212	593	305	1,492
		Slide	–	–	–	–	–	–	–
Montreal, Quebec	30	Plate	–	116	330	656	184	174	1,460
		Slide	–	40	287	439	163	119	1,048
Toledo, Ohio	19	Plate	44	272	237	436	190	108	1,277
		Slide	249	809	1,488	1,290	583	645	5,064
Richmond, Va.	27	Plate	158	206	145	195	360	213	1,277
		Slide	–	–	–	–	–	–	–
Cleveland, Ohio	18	Plate	68	145	352	252	196	226	1,239
		Slide	68	224	564	367	248	257	1,728
Milwaukee, Wis.	29	Plate	70	145	227	248	192	336	1,218
		Slide	4	2	182	80	49	205	522
Hamilton, Ontario	32	Plate	–	–	–	–	–	–	–
		Slide	–	200	591	314	103	–	1,208
Winnipeg, Manitoba	31	Plate	34	148	242	368	78	104	974
		Slide	20	61	69	223	72	35	480
Gadsden, Ala.	1	Plate	226	69	167	190	248	62	962
		Slide	86	91	65	45	65	6	358
Los Angeles, Calif.	4	Plate	248	180	122	128	136	142	956
		Slide	34	54	29	24	23	28	192
Brooklyn, N. Y.	16	Plate	–	–	–	–	–	–	–
		Slide	75	116	222	171	204	165	953
Nashville, Tenn.	25	Plate	124	216	76	64	374	60	914
		Slide	33	79	70	155	221	96	654
Youngstown, Ohio	20	Plate	–	–	–	–	–	–	–
		Slide	–	–	–	316	414	–	730
Memphis, Tenn.	24	Plate	142	66	64	86	150	35	543
		Slide	56	50	93	171	210	79	659
Savannah, Ga.	8	Plate	–	60	92	102	152	98	504
		Slide	–	–	66	71	37	35	209
Albuquerque, N. Mex.	15	Plate	40	8	65	73	180	107	473
		Slide	36	39	88	108	186	–	457
Santa Barbara, Calif.	5	Plate	66	118	113	48	41(Nov.)	30	416
		Slide	–	–	–	–	–	–	–
Dallas, Tex.	26	Plate	–	–	–	–	–	–	–
		Slide	–	–	–	256	131	17	404
Charlotte, N. C.	17	Plate	54	54	66	88	70	58	390
		Slide	–	–	–	–	–	–	–
Providence, R. I.	22	Plate	–	–	–	–	–	–	–
		Slide	–	34	106	110	58	56	364
San Diego, Calif.	6	Plate	50	52	27	27	17	30	203
		Slide	10	13	16	13	12	6	70
Tucson, Ariz.	2	Plate	28	23	42	28	38	42	201
		Slide	12	6	7	17	24	22	88
Oakland, Calif.	3	Plate	13	10	7	12	8	17	67
		Slide	37	15	16	15	8	21	112
Miami Beach, Fla.	7	Plate	–	–	–	2	–	6	8
		Slide	–	–	–	–	–	–	–

*See Fig. 1, and list of contributors on pp. 463 and 464.

is of interest because of the very low counts reported. For the entire period a total of 8 Alternaria colonies were reported. Monilia, from 1 to 3 daily, was the mold most encountered. All others occurred sporadically.

Milwaukee, Wis.: Drs. Squier and Lee reported slide and plate counts from April to Dec. 1, 1949. Alternaria and Hormodendrum comprised 18 per cent of the total plate count. (Tables I and II.) High Aspergillus counts were reported in May (470), June (390), and October (926). Counts for Monilia, Penicillium, and yeasts were also high throughout the summer months.

Montreal, Canada: Daily plate and slide counts from June 1 to November 1 were reported by Dr. Bram Rose. Alternaria and Hormodendrum comprised 25 per cent of the season's total mold count. Very high counts for Aspergilli (1,378) were reported for August; Monilia reached a peak of 1,822 in September, and yeasts counts totalling 1,946 were reported for October.

TABLE II. TOTAL HORMODENDRUM COUNT BY PLATE METHOD, MAY THROUGH OCTOBER, 1949

CITY	CONTRIBUTOR NO.*	MONTHLY HORMODENDRUM COUNT						TOTAL HORMODENDRUM
		MAY	JUNE	JULY	AUG.	SEPT.	OCT.	
Winnipeg, Manitoba	31	226	960	872	1,598	438	572	4,666
Des Moines, Iowa	13	182	1,083	615	1,038	633	218	3,769
Madison, Wis.	28	–	339	555	610	871	1,300	3,675
Los Angeles, Calif.	4	1,072	854	254	316	454	592	3,542
Nashville, Tenn.	25	502	214	6	108	1,084	908	2,822
Peoria, Ill.	11	206	315	173	317	503	375	1,889
Santa Barbara, Calif.	5	346	207	187	195	165	227	1,327
Memphis, Tenn.	24	180	35	20	263	475	150	1,323
San Diego, Calif.	6	333	202	165	195	130	256	1,281
Oklahoma City, Okla.	21	177	112	120	135	303	375	1,222
Montreal, Quebec	30	–	255	308	246	286	120	1,215
Cleveland, Ohio	18	152	145	94	178	166	428	1,163
Charlotte, N. C.	17	314	36	54	136	102	50	692
Ann Arbor, Mich.	14	47	198	18	8	235	160	666
Toledo, Ohio	19	32	50	10	60	160	282	594
Richmond, Va.	27	62	76	35	92	115	55	435
Savannah, Ga.	8	–	128	120	2	–	126	376
Johnson City, Tenn.	23	16	–	–	112	165	75	368
Gadsden, Ala.	1	19	16	62	60	82	91	330
Albuquerque, N. Mex.	15	72	34	75	50	35	50	316
Oakland, Calif.	3	49	30	22	13	28	152	294
Milwaukee, Wis.	29	–	37	42	44	72	36	231
Chicago, Ill.	9	–	–	–	3	13	112	128
Tucson, Ariz.	2	12	14	8	6	19	10	69
Miami Beach, Fla.	7	–	–	–	4	–	8	12

*See map and list of contributors.

Oakland, Calif.: This study was reported by Dr. C. L. Mauser and covered the months of April through December. Alternaria and Hormodendrum counts were low and comprised about 30 per cent of the seasonal incidence. Yeasts and unidentified molds were next in incidence and varied from 39 to 109 for each of the months with a slight rise in the fall months.

Oklahoma City, Okla.: This study by Dr. Johnny Blue was based on daily plate exposures from February 1 to November 1. Alternaria and Hormodendrum counts were high and comprised 65 per cent of the total count. The frequent Oklahoma winds resulted in an interesting graph for Alternaria with one or more spikes every month of the nine-month study. Helminthosporium and unidentified colonies were next in importance, vary-

ing from 112 to 375 a month from March to October. Counts for Aspergillus, Penicillium, Monilia, yeasts, Phoma, Mucor, and Fusarium were low and nonseasonal.

Richmond, Va.: Plate studies were made daily from April 12 to October 31, inclusive, by Dr. J. W. Thomas. Alternaria and Hormodendrum comprised 37 per cent of the total mold incidence during this period. Alternaria reached its peak in September (Table I). Yeasts, Aspergilli, Fusarium, and Monilia were of importance in that order. Helminthosporium, Phoma, and Mucor were seen infrequently.

Tucson, Ariz.: Dr. Wm. B. Steen made daily studies from May 1 to December 30, inclusive, by slide and plate methods. Yeasts comprised 67 per cent of the total seasonal count, with highest counts from September through December. Alternaria and Hormodendrum were low and together comprised less than 10 per cent of the total count. Low nonseasonal counts were also reported for Aspergillus, Penicillium, Monilia, Phoma, and Fusarium.

Salt Lake City, Utah: Dr. Dean A. Moffat reported on Alternaria spores found by the slide technique from April through September, inclusive. Counts never exceeding 1 or 2 spores daily were reported.

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Editorial

MOLDS AS ALLERGENS

The recognition of the allergenic activity of mold spores is not new, and has aided in the diagnosis and treatment of many patients with seasonal allergies. However, the opinions of allergists as to the importance of the fungi among the etiologic agents are varied, as is their utilization of mold extracts in routine testing. This is reflected in the assortments of fungus extracts offered by large manufacturers of allergens and the failure of some to include any molds in their long lists of testing materials.

The innumerable species and genera of fungi present in practically all localities has led to doubt as to number of molds which must be included in an adequate diagnostic survey. The difficulties of identification and classification of mold colonies and their spores are tremendous obstacles to all but the experienced mycologist, and precise information concerning the antigenic relationships between various species and genera is lacking.

The report of the Mold Survey Committee of the American Academy of Allergy, included in this issue, represents a cooperative effort of many workers in all parts of the United States and southern Canada to aid the allergist in dealing with these problems. This committee, headed by Dr. Leonard Harris of Northwestern University Medical School and advised by Prof. Clyde Christensen of the University of Minnesota, deserves the thanks of all interested in allergy for the accumulation of a mass of data by standard methods. The difficulties of this type of work are apparent both in the divergence of findings by different observers in the same general locality and in the lack of agreement of counts made by the slide and plate methods in many instances. However, the results are of great value in demonstrating that Alternaria and Hormodendrum are the predominant spores in the atmosphere in most parts of the territory surveyed and in disclosing the seasonal variations of incidence in various localities. It is apparent that the practicing allergist, in most parts of the country, does well to include these two molds among his routine testing materials. The reports of high concentrations of spores of other genera in certain areas are also of local interest. These findings, confirming earlier reports, help to clarify the question of exposure of the allergic patients to generally disseminated mold spores.

Many individual problems in mold sensitivity will continue to tax the ingenuity of the allergist. The damp cellar and musty summer cottage harbor molds whose spores are not a significant factor in such atmospheric surveys. Cultures of material from such sources usually demonstrate the presence of innumerable varieties of molds. Attempts to identify these and correlate their presence with skin reactions of the exposed patient offer real problems. The experiments of Prof. Christensen, also published in this issue, demonstrate graphically how readily the spores of such fungi can be spread through a house by sweeping or by handling mildewed materials.

A third and more intimate type of exposure to fungus allergens may result from the presence of saprophytic molds such as Monilia in the bronchial secretions. The demonstration of a profuse growth of such a fungus in the sputum, associated with immediate urticarial reactions to skin tests with the specific mold, may be a clue to the etiology of persistent asthma unrelated to season or environment.

Despite the progress made in the last 15 years, further clarification of the role played by molds in the causation of allergic disease should offer a fruitful field of research for years to come. Much of this investigation will require the joint resources of the clinician, the mycologist, and the chemist. The American Academy of Allergy is fortunate in having an active mold survey committee, and in having Prof. Christensen as consultant, to guide studies in this field and offer aid to the allergist who does not have access to the services of a competent mycologist.

WILLIAM B. SHERMAN

Announcements

THE AMERICAN ACADEMY OF ALLERGY
OFFERS A
POSTGRADUATE COURSE IN ALLERGY

SPONSORED BY THE

FACULTY OF MEDICINE, McGILL UNIVERSITY
OCTOBER 26, 27, 28, 1950

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Program

Thursday, October 26, Royal Victoria Hospital

9:00–10:00	Fundamental Concepts of Immunity. Antigen-Antibody Relationships E. A. Kabat, Ph.D., Columbia University
10:00–11:00	Applied Immunology in Allergic Disorders W. B. Sherman, M.D., Columbia University
11:00–12:00	Physiology of Allergic Mechanisms. Role of Histamine Bram Rose, M.D., McGill University
2:00– 3:00	The Pituitary-Adrenal Relationship J. S. L. Browne, M.D., McGill University
3:00– 4:00	ACTH and Cortisone in Relation to Allergic Mechanisms Bram Rose, M.D., McGill University
4:00– 5:00	Otorhinology in Relation to Allergy W. J. McNally, M.D., McGill University

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Friday, October 27, Royal Victoria Hospital

9:00–10:00	Patho-physiology of Allergy. Respiratory Function Tests P. Pare, M.D., K. K. Pump, M.D., and Bram Rose, M.D.
10:00–11:00	Differential Diagnosis of Asthma C. H. A. Walton, M.D., University of Manitoba
11:00–12:00	Collagen Disease G. Lyman Duff, M.D., McGill University
2:00– 3:00	Allergic Dermatosis M. B. Sulzberger, M.D., New York University
3:00– 4:00	Allergy in Children H. L. Bacal, M.D., McGill University
4:00– 5:00	Therapeutics; Antihistamines; Modified Adrenalines H. L. Mitchell, M.D., McGill University
7:00	Dinner, followed by Round Table Discussion Drs. Mitchell, Bacal, Browne, Stern, Moll, Malloy, and Rose

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Saturday, October 28, Queen Mary Veterans Hospital

9:00–10:00	Psychiatric Aspects of Allergy K. Stern, M.D., McGill University
10:00–11:00	Hay Fever J. D. L. Fitzgerald, M.D., University of Toronto
11:00–12:00	The General Adaptation Syndrome Hans Selye, M.D., University of Montreal
1:00	Luncheon

A fellowship is available in the Allergy-Arthritis Division of the Department of Internal Medicine, University of Virginia Hospital, Charlottesville, Virginia. This fellowship counts a year for Internal Medicine certification requirements. Fellows serve as consultants in Allergy and Arthritis for the Medical, Pediatrics, Dermatology and other ward services and work in the Allergy Clinic. They see private patients under a tutorial system. The stipend is $1200 per year. Room, board and laundry, etc., are not furnished.

CANADIAN SOCIETY FOR THE STUDY OF ALLERGY

VICTORIA GENERAL HOSPITAL, AUDITORIUM, HALIFAX, N. S., JUNE 20, 1950

Programme of Annual Meeting

Dermatological Allergy. Dr. K. A. Baird, West St. John, N. B.

Dandelion as a Cause of Hay-fever. Dr. H. C. Jamieson, Edmonton, Alberta.

Antibody Studies in Hay-fever in Children. Dr. S. Pedvis and Dr. H. Bacal, Montreal, Que.

Some Clinical Problems in Allergy. Dr. I. H. Erb, Toronto, Ont.

Progress in Allergy During the Past Decade. Dr. Kingsley Johnston, Dept. of Allergy, Cleveland Clinic, Cleveland, Ohio.

The Use of ACTH and Cortisone in Diseases of Hyper-sensitivity. Dr. Bram Rose, Montreal, Que.

The Effects of Phenergan on Experimental Allergic Glomerulonephritis in Rabbits. Dr. J. FitzGerald, Toronto, Ont., and Dr. J. Hamilton, Queen's University, Kingston, Ont.

Pneumothorax Occurring in Asthma. Dr. H. S. Mitchell, Montreal, Que.

SECOND INTERNATIONAL CONGRESS ON ASTHMA

MONT-DORE, PUY-DE-DÔME, FRANCE, JUNE 3 TO 5, 1950

UNDER THE PRESIDENCY OF PROFESSOR PASTEUR VALLERY-RADOT

Scientific Program

Saturday, June 3

1. Opening Address. Prof. Pasteur Vallery-Radot
2. The Future of the Question of Allergic Asthma. Dr. Duchaine, Brussels, Belgium
3. Asthma and the Heart. Prof. Agrege Lenegre and Drs. P. Maurice L. Scebat and R. Jacquot, Paris, France
4. The Present Status of the Question of Asthma in Argentina. Prof. Mariano Castex, Buenos Aires, Argentina
5. The Present Status of the Question of Asthma in Brazil. Prof. Carlos Chagas and Dr. A. O. Lima, Rio de Janeiro, Brazil
6. Asthma and the Endocrine Glands. Prof. de Gennes, Paris, France
7. Asthma and Psychic Factors. Prof. Delay, Paris, France, and Dr. Mustapha Ziwar, Alexandria, Egypt
8. The Present Status of the Question of Asthma in Spain. Prof. Jimenez Diaz, Madrid, Spain
9. The Present Status of the Question of Asthma in the United States. Dr. Francis Rackemann, Boston, Mass.
10. Radiologic Diagnosis of Asthma and of Allergic Respiratory Manifestations. Prof. Gernez-Rieux and Dr. Breton, Lille, France

Sunday, June 4

1. The Present Status of the Question of Asthma in Great Britain. Dr. Robert Coope, Liverpool, England
2. The Present Status of the Question of Asthma in Holland. Drs. E. Van Dishoeck and H. C. Olislagers, Amsterdam, Netherlands
3. Severe Asthma and Fatal Asthma. Prof. Agrege Hamburger, Paris, France
4. The Present Status of the Question of Asthma in Italy. Profs. Cesare Frugoni and Umberto Serafini, Rome, Italy
5. Asthma and the Upper Respiratory Passages. Prof. J. Rabattu, Lyon, France, and Prof. Agrege Mounier-Kuhn
6. Physio-pathology of Asthma. Prof. Santenoise, Lille, France
7. Observations on the Physio-pathology of Asthma. Prof. Paul Rossier, Zürich, Switzerland
8. Anatomic Pathology of Asthma. Prof. L. Cornil and Drs. Payan and Charpin, Marseille, France
9. Experimental Asthma. Dr. B. Halpern, Paris, France
10. Asthma and Tuberculosis. Prof. Agrege Turiaf, Paris, France

Monday, June 5

1. Infantile Asthma. Prof. G. Mouriquand and Dr. Cl. Mouriquand, Lyon, France
2. The Present Status of the Question of Asthma in the Scandinavian Countries. Dr. Ernest Salen, Stockholm, Sweden
3. The Present Status of the Question of Asthma in Yugoslavia. Prof. Spoujitch and Dr. Danilovitch, Belgrade, Yugoslavia
4. Etiologic Treatment of Asthma. Prof. P. Croizat and Drs. Lageze, Lyon, France, and Valin
5. New Methods of Treatment of Asthma. Prof. Agrege P. Milliez and Dr. Claude Laroche, Paris, France
6. Treatment of the Asthmatic State. Profs. Agreges G. Mauric and A. Domart, Paris, France
7. Asthma and Surgery. Profs. R. Leriche, Paris, France, and R. Fontaine, Strasbourg, France
8. Physical Treatment of Asthma. Dr. P. Gibert, Paris, France
9. Climate and Asthma. Dr. Blamoutier, Paris, France
10. Hydro-mineral Treatment of Asthma. Prof. Merklen, Nancy, France
11. Mont-Dore and Asthma. Dr. F. Claude

Book Reviews

THE MERCK MANUAL, ed. 8, Rahway, N. J., 1950, Merck & Co., Inc., pp. 1592. Regular edition, $4.50. Thumb-Index Edition, $5.00.

The Eighth Edition of this invaluable handbook of therapy has for the first time a separate section of 29 pages on the diseases of allergy. The general practitioner and medical student, for whom the book is designed, will find modern practical advice on symptomatic treatment of these conditions. The portion dealing with specific allergic diagnosis and treatment suffers from the brevity of discussion. The omission of any warning of the possibility of general reactions from intracutaneous tests and of any advice as to the precautions necessary in such testing is regretable. In the section which attempts to handle serum sickness and serum shock together, brevity likewise leads to confusion.

The trained allergist will find little that is new to him in the section on allergic diseases, but the remainder of the book offers an abundance of recent authoritative material on general medicine, antibiotic therapy, endocrinology, and many other subjects. The general metabolic effects of Cortisone and ACTH are discussed in a separate section and the latest available information on their therapeutic use in seventeen different diseases discussed.

It is doubtful if any other book of comparable size offers so much valuable medical information in such convenient form for reference.

PROCEEDINGS OF THE FIRST ACTH CONFERENCE HELD IN CHICAGO, OCTOBER, 1949, John R. Mote, editor, Philadelphia, 1950, The Blakiston Co., pp. 607, illustrated.

The extensive experience with ACTH in many fields reported at this conference is rich in information. Most reports are preliminary but indicate the wide therapeutic effects of this hormone. Allergy in its conventional sense is represented by Dr. Bordley and his associates from Johns Hopkins Hospital. They found encouraging responses in bronchial asthma, drug hypersensitiveness, and vasomotor alterations in the nose. Randolph and Rollins demonstrated that the clinical course of such allergic syndromes as bronchial asthma, allergic rhinitis, dermatitis, and gastrointestinal allergy, and the reduction of the clinical response following exposure to known allergens, were profoundly affected by ACTH. Rose reported a comprehensive study on eosinophilia and bronchial asthma and although his series was small, the cases were carefully investigated. Improvement occurred in all. Positive skin tests of the direct type were not altered by the hormone. The eosinophiles in the circulating blood were reduced to zero in almost all cases within 24 hours. In all cases there was a marked increase of the histidine content in the urine.

The broader aspects of allergy were represented in studies on acute glomerulonephritis, rheumatic fever, the hypersensitiveness of tuberculosis, as well as the collagen diseases which may be allied to allergy as periarteritis nodosa, disseminated lupus, scleroderma, and dermatomyositis. Here again dramatic interruption of the clinical manifestations occurred in most instances.

As in the use of ACTH and Cortisone in other diseases, all results were but temporary. One important fact that emerged from this joint study is the demonstration that adrenocorticotropic hormone is a common factor to a broad spectrum of allergic disorders and tends to link them together. The suggestion that all are due to mesenchymal effect is a stimulating one.